Development of a Degree Day Model and Economic Thresholds for Cerotoma trifurcata (Coleoptera: Chrysomelidae) in Ontario

by

Cara M. McCreary

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Environmental Biology

Guelph, Ontario, Canada

© Cara M. McCreary, August, 2013
ABSTRACT

DEVELOPMENT OF A DEGREE DAY MODEL AND ECONOMIC THRESHOLDS FOR CEROTOMA TRIFURCATA (COLEOPTERA: CHRYSOMELIDAE) IN ONTARIO

Cara Michele McCreary
University of Guelph, 2013

Advisors:
Rebecca H. Hallett
Art W. Schaafsma

Bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), is an economic pest of soybean in Ontario. Field cage studies were conducted in 2010-2011 to determine voltinism in southern Ontario and the effect of *C. trifurcata* feeding during soybean reproductive stages on soybean yield and quality. Thermal requirements for *C. trifurcata* development were determined in a laboratory study. Results of field and laboratory studies support the occurrence of one generation of *C. trifurcata* in southern Ontario. Pod-feeding increased with both number of beetles and soybean reproductive stage. An increase in defoliation and a reduction in seed quality were observed with increasing number of beetles. Monitoring programs for late-season pod-feeding should begin when degree days approach 500 (base 9.31°C). Economic thresholds for *C. trifurcata* during soybean reproductive stages range from 0.27 to 2.00 beetles per plant or 8 to 60 beetles per m of row.
Acknowledgements

It is with immense gratitude that I acknowledge my co-advisors Drs. Rebecca Hallett and Art Schaafsma for their support and guidance throughout my thesis research. Rebecca was instrumental in my career development by providing me with a background in graduate research as I worked in her lab before beginning my own studies. Rebecca’s encouragement and confidence in me throughout my research helped me develop invaluable skills allowing me to continue a career in research. Art’s irreplaceable knowledge and enthusiasm was an inspiration for my own work. Art was willing to take time to advise me on unforeseen obstacles as they arose and help me focus on my research goals.

I am truly indebted and thankful to Jocelyn Smith for her dedication and contributions. Jocelyn’s expertise and preliminary work on C. trifurcata was a valuable contribution to the success of my own research. Special thanks also goes to Christie Bahlai for her motherly guidance and her late-night statistical counseling. I would like to thank Tracey Baute from the Ontario Ministry of Agriculture and Food, for helping to locate C. trifurcata in southern Ontario and offering field assistance. I owe sincere thankfulness to my third advisory committee member, Dr. Greg Boland, for his timely advice and support.

This thesis would not have been possible without the technical and field assistance of Todd Phibbs, Jen Bruggeman, Erin LeClair, Heather Cumming, David Makynen, Braden Evans, Mike Schmidt, Kaitlyn Madge, Scott Lewis, Adam Brunke and Dave Cheung. Additional thanks to the technical staff at Ridgetown Campus for their treasured advice and moral support from Bryan Stirling, Dennis Fisher, Mike Zink and Scott Jay. I would also like to thank the co-operators that allowed access and use of their soybean fields for beetle collection and cage
studies.

Finally, I am eternally grateful to my friends, family and present colleagues for their ongoing support and encouragement. Great thanks to the late Peggy Buck, my beloved honorary Great Aunt. Peggy was the greatest motivation for my pursuit of graduate studies and was looking forward to attending my defense, but left this world too soon. I owe sincere and earnest thankfulness to my boyfriend Jeremy Pfaff, my parents and step-parents, Jim and Karen McCreary, Jackie McCreary and Barry Ashby, my in-laws Harold and Sharon Pfaff, as well as my brother and sister in-law, James and Maggy McCreary. Finally, I would like to thank my current supervisor in my position as a Research Technician in the Edible Bean Program at Ridgetown Campus, Chris Gillard, and the Edible Bean Lab, Lindsey Goudis and Ally Friesen.

Funding for this thesis was provided by the Ontario Ministry of Agriculture and Food – University of Guelph Sustainable Production (Plants) Program, and by the Grain Farmers of Ontario through the Farm Innovation Program.
Table of Contents

Acknowledgements ........................................................................................................... iii
Table of Contents .................................................................................................................. v
List of Tables ........................................................................................................................ vii
List of Figures ........................................................................................................................ ix
Table of Acronyms ................................................................................................................ xii

CHAPTER ONE: Literature Review
1.1 Introduction .................................................................................................................. 1
1.2 Classification and Distribution of Bean Leaf Beetle, Cerotoma trifurcata .................... 2
1.3 Identification .................................................................................................................. 3
1.4 Life History and Biology ............................................................................................. 4
  1.4.1 Phenology ............................................................................................................. 4
  1.4.2 Feeding Behaviour ............................................................................................... 7
  1.4.3 Overwintering Behaviour and Diapause ............................................................... 9
  1.4.4 Abundance, Dispersion and Migration ............................................................... 10
1.5 Degree Day Models ..................................................................................................... 11
1.6 Soybean Pathogens Associated with Cerotoma trifurcata ........................................ 13
  1.6.1 Bean Pod Mottle Virus Acquisition, Transmission and Symptomology ............ 13
  1.6.2 Bean Pod Mottle Virus and Soybean Mosaic Virus .......................................... 14
  1.6.3 Fungal Pathogens ............................................................................................. 15
1.7 Economic Importance ................................................................................................. 16
  1.7.1 Soybean Production ......................................................................................... 16
  1.7.2 Economic Impact of Feeding Damage ............................................................... 17
  1.7.3 Economic Impact of Pathogens ......................................................................... 18
  1.7.4 Economic Injury Level ...................................................................................... 19
1.8 Management ................................................................................................................. 21
  1.8.1 Monitoring ......................................................................................................... 21
  1.8.2 Cultural Management ....................................................................................... 22
  1.8.3 Chemical Management ...................................................................................... 23
  1.8.4 Host Plant Resistance ....................................................................................... 24
  1.8.5 Biological Control ............................................................................................. 24
  1.8.6 Pathogen Vector Management .......................................................................... 25
1.9 Research Objectives .................................................................................................... 26

CHAPTER TWO: Temperature-Dependent Development and Degree Day Requirements of
Bean Leaf Beetle, Cerotoma trifurcata, under Constant Temperature Regimes.
2.1 Abstract ...................................................................................................................... 27
2.2 Introduction ................................................................................................................ 28
2.3 Materials and Methods ............................................................................................. 29
  2.3.1 Rearing Methods ............................................................................................... 32
  2.3.2 Statistical Analysis ............................................................................................ 33
  2.3.3 Degree Day Model ........................................................................................... 34
2.4 Results ......................................................................................................................... 35
List of Tables

Table 1.1 Degree days (DD) for C. trifurcata development from egg to adult in laboratory and field settings using two lower developmental thresholds in two studies (Zeiss et al. 1996, Hammack et al. 2010). .................................................................12

Table 2.1. Growth chamber temperature conditions for C. trifurcata rearing at University of Guelph, Ontario, including coefficients for regressions of predicted¹ against observed² degree day (DD) accumulations using a minimum developmental threshold of 10.25°C. ...............................31

Table 2.2. Percentage of survivorship for each life stage event of C. trifurcata individuals reared in environmentally-controlled growth chambers under five constant temperatures at the University of Guelph, Guelph, Ontario, Canada. ........................................................................................................36

Table 2.3. Ratio of male:female adult C. trifurcata emergence from individuals reared in environmentally-controlled growth chambers under four constant temperatures at the University of Guelph, Guelph, Ontario, Canada. ........................................................................................................36

Table 2.4. Mean developmental days and rates (±SE) for life stage events of C. trifurcata reared in environmentally-controlled growth chambers under four constant temperature regimes at the University of Guelph, Guelph, Ontario, Canada. ........................................................................................................37

Table 2.5. Parameter estimates (±SE) of linear and nonlinear regressions for C. trifurcata developmental life stages under four temperatures in environmentally-controlled growth chambers at the University of Guelph, Guelph, Ontario, Canada. ........................................................................................................39

Table 2.6. Means comparison of degree day models for C. trifurcata development through life stage events while reared in growth chambers held at four temperature regimes (°C). Degree day models presented are LS means (±SE) of actual degree day accumulations (base 11.58 and 10.25°C) calculated for 30 min. intervals for each temperature regime and the estimated degree days calculated from the inverse of the slope (b) of the linear regression. ........................................41

Table 3.1. Summary of infestation dates, sampling completion dates and number of male and female C. trifurcata used for field cage trials at 5 locations over 2 years........................................55

Table 3.2. Summary of temperature records used in analyses of C. trifurcata development after field cage trials at 5 locations over 2 years.........................................................58

Table 3.3. Mean number of developmental days (± SE) and mean degree day accumulations (± SE) (base 10.25°C) between C. trifurcata life stage events in field cage trials at Bruce, Huron and Wellington counties and the Municipality of Chatham-Kent in 2010 and 2011. ..................................67

Table 3.4. Comparison of observed mean degree day requirements (base 10.25°C) versus predicted estimates for C. trifurcata life stage events for egg hatch and egg hatch to pupation and egg deposit to peak adults in Ontario soybean fields based on sampling from pan and canopy
traps in 2009 in Auburn and Benmiller (Brunke 2011), and field cage trials in 2010 and 2011 in Ridgetown.

Table 4.1. Mean (±SE) 100-seed weight, percentage of oil and protein content across all levels of beetle treatments (0, 2, 4, 8, 16 beetles per plant and an uncaged control) from *C. trifurcata* field cage studies during soybean pod-formation in Ridgetown, ON, Canada, 2010-11.

Table 4.2. Regression coefficients for number of beetles per plant and number of feeding days against harvested seed quality grading categories from *C. trifurcata* field cage studies with R-stage treatments combined during soybean pod formation in Ridgetown, ON, Canada, 2010-11.

Table 4.3. Mean percentage of seed (± 95% CI) in graded quality categories after *C. trifurcata* field cage studies during soybean pod-formation (R3-R6) in Ridgetown, ON, Canada, 2011.

Table 4.4. Mean percentage of seed (± 95% CI) in graded quality categories after *C. trifurcata* cage infestations during soybean pod-formation (R3-R6) in Ridgetown, ON, Canada, 2011.

Table 4.5. Mean percentage of seed (± 95% CI) with a significant effect from soybean reproductive stage (R3-R6) during field cage studies of *C. trifurcata* in Ridgetown, ON 2010-2011.

Table 4.6. Economic injury levels (i.e. number of beetles per plant or metre of row) for *C. trifurcata* during pod-formation on soybeans for a range of soybean market values and pest management costs.
List of Figures

Figure 1.1  Distribution of the number of generations documented for *C. trifurcata* in the United States 6

Figure 2.1. Temperature-dependent development for *Cerotoma trifurcata* reared in environmentally-controlled growth chambers under four constant temperature regimes (18.8, 23.4, 26.1, 30.9°C) at the University of Guelph, Guelph, Ontario, Canada. (A) Eclosion. (B) Pupation. (C) Adult emergence. (D) Egg deposit to adult emergence. Solid line represents the fitted physiological growth curve. Dotted line represents the linear regression. Circles represent means from linear regression 38

Figure 2.2. Relative frequency histograms and cumulative percent distributions of *C. trifurcata* degree day accumulations to eclosion (base 10.25°C) after rearing in growth chambers under four constant temperature regimes (°C) 42

Figure 2.3. Relative frequency histograms and cumulative percent distributions of *C. trifurcata* degree day accumulations to pupation (base 10.25°C) after rearing in growth chambers under four constant temperature regimes (°C) 43

Figure 2.4. Relative frequency histograms and cumulative percent distributions of *C. trifurcata* degree day accumulations to adult emergence (base 10.25°C) after rearing in growth chambers under four constant temperature regimes (°C) 44

Figure 2.5. Relative frequency histograms and cumulative percent of distributions of *C. trifurcata* degree day accumulations from egg deposit to adult emergence (base 10.25°C) after rearing in growth chambers under four constant temperature regimes (°C) 46

Figure 3.1. Number of *C. trifurcata* generations in the United States and field study site locations in southwestern Ontario in 2010 and 2011. Ontario study site locations: 1) Bruce county 2010/Huron county 2011, 2) Wellington county 2010, 3) Municipality of Chatham-Kent 53

Figure 3.2. Abundance of *C. trifurcata* eggs, larvae and pupae per cm³ soil and teneral adults per cage at three locations in southwestern Ontario in 2010 from field cage trials in soybean fields 61

Figure 3.3. Abundance of *C. trifurcata* eggs, larvae and pupae per cm³ soil at two locations in southwestern Ontario in 2011 from field cage trials in soybean fields 62

Figure 3.4. Immature *C. trifurcata* recovered from soil samples after adults were confined in field cage studies in three counties in southwestern Ontario in 2010. Sample photographs are (A) hatched eggs and (B) unhatched eggs from the Bruce County site on 13 July, 2010; (C) larva from the Wellington site on 2 August, 2010, and (D) pupa from the Wellington site on 16 August, 2010. Photographs were taken by Heather Cumming and Dave Cheung in the University of Guelph Insect Systematics Lab 63
Figure 3.5. Abundance of larval instars per cm³ based on head capsule size after field cage trials at five locations in 2010 and 2011. Head capsule ranges for first, second and third instars were: 0.20-0.28, 0.30-0.40 and 0.42-0.52 mm, respectively .................................................................65

Figure 3.6. Linear regression and observed larval head capsule width (mm) from specimens recovered from soil samples after C. trifurcata field cage trials at three locations in Ontario in 2010 and 2011 ..........................................................................................................................66

Figure 3.7. Linear regressions of degree day accumulations using different lower developmental thresholds (base temperatures) calculated from temperature data at five sites from 7 July to 15 September in southwestern Ontario in 2010-2011. Lower developmental thresholds used were based on those determined from linear regressions for development of C. trifurcata life stage events (Chapter 2) and all were regressed against the best estimate determined from the temperature-dependent development study (10.25°C) (Chapter 2). (A) Base 9.25°C for pupation; (B) Base 9.47°C for adult emergence; (C) Base 9.31°C for egg deposit to adult emergence .......68

Figure 3.8. Linear regressions of degree day accumulations using the standard base temperature used for soybean heat units against the best estimate from the temperature-dependent development study (10.25°C) (Chapter 2) ........................................................................................................71

Figure 4.1. Mean percentage of defoliation (± 95% CI) from field cage studies of adult C. trifurcata during pod development of soybeans in Ridgetown, ON, 2010-11. Treatments with different numbers of beetles were pooled to examine the effect of reproductive stage at time of treatment. Arcsine square root transformations were used for analyses to satisfy assumptions of normality based on the highest Shapiro-Wilk statistic. Back-transformed values are presented. Columns within the same year with the same letter are not significantly different (P ≥ 0.05); lower case: 2010; upper case: 2011 ........................................................................................................................................83

Figure 4.2. Mean percentage of defoliation (± 95% CI) from field cage studies of adult C. trifurcata during pod development of soybeans in Ridgetown, ON, 2010-11. Treatments were 0, 2, 4, 8, 16 beetles per plant and an uncaged control. Timing based on reproductive stage were pooled to examine the effect of number of beetles. Arcsine square root transformations were used for analyses to satisfy assumptions of normality based on the highest Shapiro-Wilk statistic. Back-transformed values are presented. Columns within the same year with the same letter are not significantly different (P ≥ 0.05); lower case: 2010; upper case: 2011 ........................................................................................................................................84

Figure 4.3. Mean percentage of pods with feeding damage (A), number of lesions per pod (B), number of lesions per plant (C), and number of stem lesions per plant (D) from C. trifurcata field cages studies during soybean reproductive stages (R3-R6) in Ridgetown, Ontario, Canada, 2010. To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, percentage of pods with feeding damage, number of lesions per pod and number of pod lesions per plant were arcsine square root transformed, and number of lesions per stem was natural log transformed for the analyses. Means presented here are back-transformed to the original scale ..85

Figure 4.4. Mean of percentage of pods with feeding damage (A), number of lesions per pod (B), number of lesions per plant (C), and number of clipped pods per plant (D) from C. trifurcata
field cage studies during soybean reproductive stages (R3-R6) in Ridgetown, Ontario, Canada, 2011. To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, number of lesions per pod were square root transformed, number of clipped pods per plant were arcsine square root transformed, and percentage of pods with feeding damage and number of pod lesions per plant were natural log transformed for the analyses. Means presented here are back-transformed to the original scale.

Figure 4.5. Mean (± 95% CI) number of stem lesions per plant based on (A) soybean reproductive stage and (B) number of beetles per plant from C. trifurcata field cage studies during soybean reproductive stages in Ridgetown, Ontario, Canada, 2011. To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, number of stem lesions per plant were natural log transformed for the analysis. Means presented here are back-transformed to the original scale.

Figure 4.6. Mean (± SE) yield after field cage studies of adult C. trifurcata during pod development of soybean reproductive stages in Ridgetown, ON, 2010-11. Treatments with different numbers of beetles were pooled to examine the effect of reproductive stage at time of treatment. Columns within the same year with the same letter are not significantly different (P ≥ 0.05); lower case: 2010; upper case: 2011.

Figure 4.7. Mean (± SE) yield from field cage studies of adult C. trifurcata during pod development of soybeans in Ridgetown, ON, 2010-11. Treatments were 0, 2, 4, 8, 16 beetles per plant and an uncaged control. Timing based on reproductive stage were pooled to examine the effect of number of beetles. Columns within the same year with the same letter are not significantly different (P ≥ 0.05); lower case: 2010; upper case: 2011.

Figure 4.8. Linear regression of yield reductions (%) from C. trifurcata field cage studies during reproductive soybean stages (R3-R6) in 2011, Ridgetown, ON, Canada. Yield reductions were calculated as a percentage reduction from the caged control for each replicate in a split-plot design, with R-stage as the main effect and number of beetles (0, 4, 8, 16 and uncaged control) per plant as the sub-plot effect.

Figure 4.9. Linear regression of 100-seed weight and percentage of defoliation from C. trifurcata field cage studies (2010: 0, 2, 4, 8 beetles per plant and an uncaged control; 2011: 0, 4, 8, 16 beetles per plant and an uncaged control) during reproductive soybean stages (R3-R6) in 2011, Ridgetown, ON, Canada.

Figure 4.10. Percentage of (A) mouldy white and (B) shrivelled seed from C. trifurcata field cage studies in Ridgetown, ON, Canada, 2011. A significant interaction was observed between the number of beetles per plant and soybean reproductive stage. Percentage of mouldy white seed was arcsine square root transformed and shrivelled seed was log transformed for analysis. Back-transformed means are presented.

Figure 4.11. Linear regression of yield and number of beetles per plant from C. trifurcata field cage studies (2010: 0, 2, 4, 8 beetles per plant; 2011: 0, 4, 8, 16 beetles per plant) during reproductive soybean stages (R3-R6) in 2011, Ridgetown, ON, Canada.
Table of Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIC</td>
<td>Akaike information criterion</td>
</tr>
<tr>
<td>BPMV</td>
<td>bean pod mottle virus</td>
</tr>
<tr>
<td>CHU</td>
<td>crop heat units</td>
</tr>
<tr>
<td>CI</td>
<td>confidence intervals</td>
</tr>
<tr>
<td>DD</td>
<td>degree day</td>
</tr>
<tr>
<td>ET</td>
<td>economic threshold</td>
</tr>
<tr>
<td>EIL</td>
<td>economic injury level</td>
</tr>
<tr>
<td>IPM</td>
<td>integrated pest management</td>
</tr>
<tr>
<td>R-stage</td>
<td>soybean reproductive stage</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SMV</td>
<td>soybean mosaic virus</td>
</tr>
</tbody>
</table>
CHAPTER ONE

Literature Review

1.1 Introduction

In Ontario, several insects are known economic pests of soybeans, *Glycine max* (L.) Merr. (Fabaceae), and infestations can cause economic losses to soybean growers. Among these is the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae) (Chittenden 1897). Pest management programs require proper identification and understanding of life history, population dynamics, and ecology of the pest (Norris et al. 2003). Each of these factors influences management strategy decisions and the timing of monitoring and control actions. *C. trifurcata* can cause direct damage by feeding on plant parts and enhance secondary damage from viral and fungal pathogens (Shortt et al. 1982, Smelser and Pedigo 1992b, c, Hunt et al. 1995, Bradshaw et al. 2007). Although it is a native insect, and economically damaging populations have occurred for over a century in the United States of American (US), *C. trifurcata* is a relatively new concern for growers in Ontario (A. W. Schaafsma, A. Tenuta, personal communications 1).

Since the early 2000’s, an apparent increase in abundance of *C. trifurcata* in Ontario has caused concern about greater damage to soybean crops. In the US, where most research has been undertaken, *C. trifurcata* feeding damage can cause up to 50% yield and quality loss; however, losses can increase to 80% when pathogen infection occurs as a result (Ross 1968, Smelser and

---

1 A. W. Schaafsma, Department of Plant Agriculture, University of Guelph Ridgetown Campus, Ridgetown, Ontario, Canada; A. Tenuta, Field Crop Pathologist, Ontario Ministry of Agriculture Food and Rural Affairs, Ridgetown, Ontario, Canada.
Pedigo 1992c). Little is known about the distribution, frequency, biology and economic impact of *C. trifurcata* in Ontario.

Methods used to assess and control this pest need to be developed and applied specific to the environmental and agronomic conditions of Ontario (Walter 2003). Successful management programs are often replicated in new contexts, without attempt for further understanding of the pest species in new localities or its associations with new hosts (Walter 2003). An understanding of *C. trifurcata* biology in Ontario is necessary to build successful management strategies for growers. Current knowledge on the life history, ecology and management approaches for this species was reviewed to help inform investigations of the control and management of *C. trifurcata* in Ontario.

1.2 **Classification and Distribution of Bean Leaf Beetle, *Cerotoma trifurcata***

*C. trifurcata* belongs to the Chrysomelid tribe Luperini, as do several important related pest species, including: northern corn rootworm, *Diabrotica longicornis* (Say) or *D. barberi* Smith & Lawrence, western corn rootworm, *D. virgifera virgifera* LeConte, and spotted cucumber beetle, *D. undecimpunctata howardi* Barber. *C. trifurcata* feed on plants primarily in the family Fabaceae, and were first described by Forster as a North American species (Forster 1771). In the late 1800’s, *C. trifurcata* was reported on several species of leguminous crops in the US as far north as Ohio (Popenoe 1877, Chittenden 1897), and is found throughout North America from southern Canada to the Gulf of Mexico (Chittenden 1897, McConnell 1915, Eddy and Nettles 1930, Isely 1930). Anecdotal reports indicate the first known presence of *C. trifurcata* occurred in southwestern Ontario in the 1970’s, with first evidence of overwintering populations observed in the early 2000’s (A. W. Schaafsma, A. Tenuta, personal
communications²).

1.3 Identification

*C. trifurcata* can be distinguished from other beetles belonging to the same tribe. While *Diabrotica longicornis*, *D. barberi*, and *D. virgifera virgifera* are found primarily on maize, *Zea mays* L., *D. undecimpunctata howardi* is known to feed on legumes (Fischer et al. 1990, Wang et al. 1994). Adults of *C. trifurcata* may reach 5 mm in length, with their most distinguishing feature an inverted black triangle behind the scutellum (Isely 1930). They are quite variable in colour and spotting, ranging from pale yellow or tan to a deep red, with 0 to 6 black spots on the elytra (Chittenden 1897, Isely 1930). Sexual dimorphism occurs in *C. trifurcata*; the most obvious character visible to the naked eye is that the frons is yellow or white in males, while in the female it is black (Isely 1930, Ruppel 1971). Additionally, males are differentiated by a hairless patch on their prothoracic legs (Hammack and French 2007).

*C. trifurcata* eggs are orange and spindle-shaped, less than 1 mm in length, with a coarsely reticulated surface (McConnell 1915, Isely 1930). Larvae are slender, white with a black head and anal shield and reach a maximum of 10 mm in length (Chittenden 1897, McConnell 1915, Isely 1930). The 3 instars can be differentiated by head capsule width: L1: 0.17-0.19 mm; L2: 0.27-0.30 mm; and L3: 0.40-0.42 mm (Isely 1930). The pupae are translucent, less than 5 mm in length, with visible elytra and legs, and with 2 caudal spines at the base of the abdomen (Isely 1930).

_____________________

² A. W. Schaafsma, Department of Plant Agriculture, University of Guelph Ridgetown Campus, Ridgetown, Ontario, Canada; A. Tenuta, Field Crop Pathologist, Ontario Ministry of Agriculture Food and Rural Affairs, Ridgetown, Ontario, Canada.
1.4 Life History and Biology

1.4.1 Phenology

The life cycle of *C. trifurcata* begins with an overwintering adult generation (F₀) which emerges from woodlots and leaf litter in spring to feed on alfalfa or wild legumes (Smelser and Pedigo 1991, Krell et al. 2003, Hammack et al. 2010) before colonizing, or populating, newly emerged soybean fields (Waldbauer and Kogan 1976, Helm et al. 1983, Loughran and Ragsdale 1986b, Witkowski and Echtenkamp 1996, Obopile and Hammond 2001). Depending on location, overwintering adults emerge from early April to mid-May (Isely 1930, Helm et al. 1983, Jeffords et al. 1983, Loughran and Ragsdale 1986b), with time of emergence corresponding to day-length and temperature. Post-overwintering emergence begins from day-lengths of 13 h in North Carolina to 14.5 h in southern Minnesota, under average daily temperatures of 26°C, 17-18.5°C, and 15°C, in North Carolina (Boiteau et al. 1979b), Minnesota (Loughran and Ragsdale 1986b), and Illinois (Jeffords et al. 1983), respectively.

Mating and ovarian development begins in alfalfa fields, where >60% of females were found to be inseminated, compared with <3% of females in overwintering sites (Loughran and Ragsdale 1986b, Hammack et al. 2010). When adults colonize a soybean field, they begin feeding on soybean seedlings and females oviposit in the soil at the base of host plants (McConnell 1915, Eddy and Nettles 1930, Isely 1930, Waldbauer and Kogan 1976, Mabry et al. 2003). Although eggs have also been found in soil surrounding alfalfa plants, there is no evidence of a complete life cycle occurring on alfalfa (Zeiss and Pedigo 1996).

The location and quantity of eggs in the soil surrounding soybean plants have been efficiently and accurately sampled using a modified Illinois egg separator technique (Waldbauer and Kogan 1973, 1975). Oviposition occurs within a 5-cm radius of the base of the plant.
(Waldbauer and Kogan 1973), although 77% of eggs are found within 2.5 cm of a plant and 93% within 7.6 cm of a row (McConnell 1915, Waldbauer and Kogan 1973, 1975, Levinson et al. 1979). Eggs are deposited on the base of the stem, in crevices and beneath the soil (McConnell 1915) at depths up to 3.8 cm (Waldbauer and Kogan 1975). Eggs laid by colonizing females tend to be aggregated, while eggs from successive summer generations are randomly distributed (Levinson et al. 1979).

Larvae are found in close proximity to roots (Isely 1930, Anderson and Waldbauer 1977, Levinson et al. 1979) with 92% located in the top 7.6 cm of soil (Anderson and Waldbauer 1977, Kogan and Herzog 1980) where they sometimes burrow into rhizobium nodules of leguminous plants (Marrone and Stinner 1984). Sampling techniques for larvae have been developed similar to those used for egg extraction, but modified to suit the size and fragility of larvae and pupae (Anderson and Waldbauer 1977, Levinson et al. 1979). Few larvae and pupae were found further than 23 cm laterally from a row of host plants (Levinson et al. 1979).

Pupation occurs in the soil in oval cells formed by fully grown larvae (Isely 1930), at a depth of approximately 10 cm (Isely 1930), although dry conditions can cause C. trifurcata to burrow deeper to pupate (McConnell 1915). Adult emergence is followed by several days of heavy feeding before mating and oviposition begin (Isely 1930).

The number of generations varies from one to three per year throughout its North American range (Fig. 1.1) (McConnell 1915, Isely 1930, Waldbauer and Kogan 1976, Loughran and Ragsdale 1986b, Smelser and Pedigo 1991, Witkowski and Echtenkamp 1996, Danielson et al. 2000, Obopile and Hammond 2001, Hammack et al. 2010). However, the number of generations in Ontario is unknown. Successful management requires knowledge of the number of generations and population growth patterns (Norris et al. 2003).
Although environmental conditions impact the rate of larval development and the timing of the first summer generation (F₁) emergence (Kogan et al. 1974, Marrone and Stinner 1984), teneral adults are commonly seen during early reproductive stages of soybeans (Loughran and Ragsdale 1986b, Witkowski and Echtenkamp 1996, Hammack et al. 2010). Presence of a second summer generation (F₂) is typically evidenced by a second peak in the adult population (Waldbauer and Kogan 1976, Witkowski and Echtenkamp 1996, Lam et al. 2001, Hammack et al. 2010) which can continue through soybean senescence (Smelser and Pedigo 1991). A third summer generation (F₃) also occurs in the most southern states (McConnell 1915, Isely 1930). Regardless of the number of generations, the last summer generation overwinters and little to no mating and oviposition occurs prior to winter (Hammack et al. 2010).

1.4.2 Feeding Behaviour

Early accounts from the US reported *C. trifurcata* causing significant damage to a wide variety of beans, including several varieties of *Phaseolus vulgaris* L. (common bean, string bean, etc.), *P. lunatus* L. (lima bean), *Vigna unguiculata* (L.) Walp (cowpea), as well as several wild leguminous hosts (Chittenden 1897, Eddy and Nettles 1930, Isely 1930). By the early 1900’s, many new host plants had been documented as food plants, including soybeans (McConnell 1915). In addition, *C. trifurcata* has been observed to feed on at least a dozen other commercially grown and wild legumes; the most commonly reported are *Lespedeza* spp., *Desmodium* spp., *Meibomia* spp., *Phaseolus* spp., and *Trifolium* spp. (Chittenden 1897, McConnell 1915, Isely 1930, Bradshaw et al. 2007). Although overwintering adults will feed on *Medicago sativa* L. or *M. ruthenica* (L.) Trautv., (alfalfa) foliage before colonizing soybean (Zeiss and Pedigo 1996), soybean is the preferred host (Isely 1930, Helm et al. 1983, Koch et al. 2004).
Abundance of alternate hosts before soybeans emerge may influence feeding choices made by the beetle. *C. trifurcata* adults have been observed to feed and aggregate on nonleguminous hosts, with *Urtica dioica* L. (stinging nettle), *Laportea canadensis* (L.) Wedd. (Canadian woodnettle), and *Euonymus atropurpurea* Jacq. (eastern wahoo) confirmed as acceptable food plants (Helm et al. 1983). In central Illinois, where these observations were made, leguminous hosts were scarce by comparison with nonleguminous plants found in the understory and along edges of woodlots (Helm et al. 1983). In the midwestern US where maize (*Zea mays* L.) predominates, *C. trifurcata* is reported to feed on young maize plants (Zeiss and Pedigo 1996). More recently, *C. trifurcata* was observed feeding in a commercial pumpkin field and further testing confirmed that adults will feed on several curcurbit species (Cucurbitaceae) (Koch et al. 2004). In addition to general abundance of potential food plants, other factors such as nutritional acquisition may influence host plant choice as seen in the ability of other chrysomelid species to choose alternate suitable host plants when the preferred nitrogen-rich host is unavailable (Obermaier and Zwölfer 1999).

Adult *C. trifurcata* feed on leaves, stems, pods and peduncles of soybean (Chittenden 1897, Leonard and Turner 1918, Isely 1930, Kogan and Herzog 1980, Smelser and Pedigo 1992b, c). Outer layers of the pod are consumed down to the endocarp, exposing seeds and increasing the possibility of pathogen infection (Kogan and Herzog 1980, Shortt et al. 1982, Smelser and Pedigo 1992b). Peduncles are weakened by feeding and the entire pod may be lost as a result (Smelser and Pedigo 1992b, c).

Cage studies revealed that few adult beetles feed for longer than 21 days (Smelser and Pedigo 1992b). Estimates of consumption rates range from 0.31 to 1.00 cm² leaf tissue per d (Waldbauer and Kogan 1976, Hunt et al. 1995). Lower rates of leaf tissue consumption may be
the result of increased feeding on pods and stems (Smelser and Pedigo 1992b), as pod injury increased from 4 to 25% during pod-fill stage soybeans (reproductive stages R4 to R6) (Fehr et al. 1971, Obopile and Hammond 2001).

### 1.4.3 Overwintering Behaviour and Diapause

Overwintering generally begins in late September or early October with post-harvest flights to overwintering sites containing as much as 75% females (Jeffords et al. 1983). However, in Minnesota, where one summer generation occurs, adult *C. trifurcata* have been known to move to overwintering sites as early as late July and mid-August (Payah and Boethel 1985, Loughran and Ragsdale 1986b). Over 99% of overwintering beetles remain in soybean fields or enter woodlots (Lam and Pedigo 2002) and the remainder overwinter in alfalfa or corn fields, grasslands and ditches (Chittenden 1897, McConnell 1915, Isely 1930). Overwintering sites may be slightly elevated to avoid excess water (McConnell 1915). Through the winter beetles tend to remain near the edge of overwintering sites beside agricultural fields (Jeffords et al. 1983).

Winter survival can influence abundance of colonizing populations and distribution of *C. trifurcata* throughout North America. Overwintering success in colder climates, like Minnesota (Carrillo et al. 2005) or southern parts of Canada, may be improved by snow cover insulation (Hart and Lull 1963). Predictive models have been developed to assess timing of emergence and winter survivorship (Carrillo et al. 2005). Winter mortality in Minnesota was between 41 and 66% (Carrillo et al. 2005), whereas in Louisiana mortality varied from 54 to 82% (Payah and Boethel 1985) and in central Iowa from 49 to 89% (Lam and Pedigo 2000). Results from cold bath treatments indicate that significant mortality occurs at ambient temperatures between -5 and -10°C, while daily mean temperatures in leaf litter generally stayed above -5°C (Lam and Pedigo
Mating and ovarian development rarely occur in overwintering females (Smelser and Pedigo 1991), with no developing oocytes found in females collected in the fall (Hammack et al. 2010). Reproductive diapause, which also occurs in its close relative, *D. undecimpunctata howardi*, is induced by short day lengths of 12 and 13 h at temperatures of 20 and 25°C, although warmer temperatures can delay this induction (Elsey 1988). In Iowa, no egg development was found in females collected in mid- to late August (Smelser and Pedigo 1991). In eastern South Dakota, when day length was <14 h, reproductive behaviours were terminated (Hammack et al. 2010). Induction of early reproductive diapause in *C. trifurcata* may influence the number of generations that occur at a given locale.

1.4.4 Abundance, Dispersion and Migration

Although *C. trifurcata* is rarely seen in flight (Chittenden 1897), their movement in and amongst soybean fields and overwintering sites has been monitored using Malaise and sticky-board traps (Jeffords et al. 1983). Flight patterns are random (Jeffords et al. 1983) and aggregations of adults within soybean fields have been observed (Smelser and Pedigo 1992a). Sudden declines in populations may be attributed to mortality or migration (Hammack et al. 2010). However, aside from late-season movement into younger host fields after senescence has begun (Pedigo and Zeiss 1996), adults rarely move from one soybean field to another (Waldbauer and Kogan 1976).

Precipitation and temperature throughout the growing season influences survivorship of *C. trifurcata* (Jeffords et al. 1983, Lam et al. 2001). Extreme dryness inhibits eggs from hatching and may cause desiccation of larvae (Marrone and Stinner 1983, 1984). F2 beetle populations were greater after overwintered adult emergence was well synchronized with
soybean planting followed by a warmer growing season in 1980 than in 1981 in Illinois (Jeffords et al. 1983). Soil type may also contribute to population densities of *C. trifurcata*. The eastern coastal plain of North Carolina, which is characterized by organic and mineral-organic soils, has the largest populations of *C. trifurcata* in the state (Marrone and Stinner 1983, 1984). Clay content in soil, especially during dry periods when the soil surface hardens, may prevent larvae and pupae from completing development (Kogan et al. 1974, Marrone and Stinner 1984). Larval development was shortest and survival was greatest in wet and organic soils (Marrone and Stinner 1984).

Synchronization of soybean phenology and *C. trifurcata* development plays a major role in the insect’s abundance (Helm et al. 1983) given that this beetle has a strong preference for a single food source. For example, in Illinois, emigration from overwintering sites was delayed by cool conditions until most soybean fields were planted, which led to large populations throughout the growing season, while a warm spring accelerated beetle dispersal and resulted in small populations (Jeffords et al. 1983).

1.5 **Degree Day Models**

Proper timing of monitoring and application of management tactics is crucial to the success of pest management programs. Development of *C. trifurcata*, as with many other insects and plants, is at least partially dictated by the accumulation of heat, or degree days (DD), as temperature influences biochemical reactions (Pedigo and Rice 2009). Degree day models can be developed to predict when life stages or generations of insects will occur and can be better predictors than calendar days (Pruess 1983, Pedigo and Rice 2009). Degree day models for *C. trifurcata* development have been developed (Zeiss et al. 1996, Hammack et al. 2010), but
Table 1.1 Degree days (DD) for *C. trifurcata* development from egg to adult in laboratory and field settings using two lower developmental thresholds in two studies (Zeiss et al. 1996, Hammack et al. 2010).

<table>
<thead>
<tr>
<th>Location</th>
<th>DD ± SE Field</th>
<th></th>
<th>DD ± SE Lab</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base 7.61°C</td>
<td>Base 11.58°C</td>
<td>Base 7.61°C</td>
<td>Base 11.58°C</td>
</tr>
<tr>
<td>Ames, IA</td>
<td>673.6 ± 28.7¹</td>
<td>495.1 ± 19.6¹</td>
<td>646.4 ± 17.4⁵</td>
<td>491.0 ± 8.1⁵</td>
</tr>
<tr>
<td></td>
<td>740.0 ± 58.2²</td>
<td>541.6 ± 42.5²</td>
<td>597.7</td>
<td>398.2³</td>
</tr>
<tr>
<td>Brookings, SD</td>
<td>540.4³</td>
<td>383.3³</td>
<td></td>
<td>597.7¹</td>
</tr>
</tbody>
</table>

¹First generation, ²Second generation, ³Year 1, ⁴Year 2, ⁵Larvae fed soybean roots, ⁶Larvae fed cowpea cotyledons.
SE represents standard error of the mean.
discrepancies exist between results from different studies and between those conducted in the laboratory and the field (Table 1.1). Various minimum developmental thresholds used to calculate degree days and predict life stage events are reported for *C. trifurcata* (Pedigo and Rice 2009). Estimates for minimum developmental thresholds for *C. trifurcata* are reported to be 11.58°C and 7.61°C for beetles reared on cowpea cotyledons and soybean roots, respectively (Zeiss et al. 1996). However, difficulty in regulating moisture for those reared on soybean roots resulted in high mortality and much lower sample size than beetles fed cowpea cotyledons; therefore, 11.6°C was recommended as the more reliable threshold (Zeiss et al. 1996). When using degree days to predict emergence of overwintered beetles from leaf litter, base 7.5°C was the best predictor (Jeffords et al. 1983). Standardized thresholds should be used (Pruess 1983) and more research is necessary to determine the appropriate minimum developmental threshold for *C. trifurcata*.

1.6 Soybean Pathogens Associated With *Cerotoma trifurcata*

1.6.1 Bean Pod Mottle Virus Acquisition, Transmission and Symptomology

Bean pod mottle virus (BPMV), *Comovirus* (Comoviridae), is a virus of soybean and other legumes (Giesler et al. 2002), first detected in 1948 on *Phaseolus vulgaris* L. var. Tendergreen and first identified as a soybean pathogen in Arkansas in 1951 (Walters 1958, Giesler et al. 2002). BPMV occurs throughout the southern and southeastern US in soybean production areas (Horn et al. 1973, Giesler et al. 2002). Virus-contaminated *C. trifurcata* were found in Ontario in 2000, but BPMV was not positively identified in the field until 2001 in samples from soybean breeding nurseries in Essex and Kent counties (Michelutti et al. 2002).

BPMV is vectored primarily by *C. trifurcata* (Ross 1963, Patel and Pitre 1976, Kogan
and Herzog 1980, Hopkins and Mueller 1984, Giesler et al. 2002, Bradshaw et al. 2007), However, other leaf-feeding beetles are capable of acquiring and transmitting the virus (Patel and Pitre 1971). Alternate hosts, such as wild legumes, are potential sources of viral inoculum if beetles feed on them prior to colonizing soybeans (Krell et al. 2003, Bradshaw et al. 2007). The virus has been detected from beetle extracts using enzyme-linked immunosorbant assays (ELISA) (Ghabrial and Schultz 1983). BPMV titers from beetles were higher than those from soybean leaf extracts but declined over time (Ghabrial and Schultz 1983). Despite BPMV detection in seed, plants grown from infected seed typically fail to show symptoms of infection (Schwenk and Nickell 1980).

Symptoms of BPMV on soybean include foliar mosaic, mottling of seeds and pods, stunting and necrosis (Ross 1968, Giesler et al. 2002, Hobbs et al. 2003, Zheng et al. 2005b). BPMV is thought to be one of the causes of “green stem” which delays maturation of soybean stems and can result in necrosis and death of the plant (Schwenk and Nickell 1980). A field study in Manhattan, KS, noted that when the virus was detected, there would typically be high infection rates throughout the crop and symptoms were often still present late in the season (Schwenk and Nickell 1980). BPMV infection can reduce seed pod numbers and reduce plant height by as much as 80% (Schwenk and Nickell 1980). Seed mottling is an unreliable indicator of viral infection because the BPMV antigen has also been detected in non-mottled seed (Krell et al. 2005). The major concern about BPMV for soybean growers would be its effect on quality of food grade seed, which must be symptom-free for export, and on the overall health of infected plants.

1.6.2 Bean Pod Mottle Virus and Soybean Mosaic Virus

Soybean mosaic virus (SMV), Potyvirus (Potyviridae), has similar symptoms to BPMV
(Ross 1968, 1969, Giesler et al. 2002), but is vectored primarily by the soybean aphid, *Aphis glycines* Matsumura, an invasive insect pest of soybeans that feeds on sap of leaves, stems, flowers and pods (Ragsdale et al. 2004). As late-season pod-feeding by *C. trifurcata* begins in late August and large populations of *A. glycines* have been observed in mid- to late August (Johnson et al. 2008), it is possible for the two pests, and consequently the two viruses, to occur simultaneously. Previous work suggests there is an additive effect between BPMV and SMV (Ross 1968, Tu et al. 1970, Hobbs et al. 2003), compared with BPMV alone (Hopkins and Mueller 1984). In addition, when BPMV was present, increased symptom severity (Anjos et al. 1992), reductions in seed size and seedling emergence, and increased mottling by SMV occurred (Ross 1968, 1969). Increased titer of BPMV was detected when soybean plants were also infected with SMV, compared with plants infected with BPMV alone (Anjos et al. 1992).

### 1.6.3 Fungal Pathogens

Several plant-pathogenic fungi have been isolated from adult beetles, suggesting that beetles may play a role in spreading these pathogens to plants (Shortt et al. 1982). Secondary infections from fungal pathogens may result from tissue exposed by pod-feeding (Smelser and Pedigo 1992c). In Ohio, *Alternaria tenuissima* (Kunze) Wiltshire, was the most prevalent fungal pathogen recovered beneath pod lesions caused by *C. trifurcata* feeding (Obopile and Hammond 2001). In Illinois, *A. tenuissima, A. alternata* (Fr.) Keissl and eight other species of fungi were isolated from the heads and abdomens of adult *C. trifurcata* and are thought to be borne internally (Shortt et al. 1982).

Symptoms caused by fungal pathogens include shrunken, distorted and discoloured seed (Obopile and Hammond 2001) and reduced seed viability (Shortt et al. 1982). *A. tenuissima* was not isolated from non-injured pods (Shortt et al. 1982), suggesting that *C. trifurcata* was a likely
candidate for transmission.

1.7 Economic Importance

1.7.1 Soybean Production

Soybean belongs to the family Fabaceae (legumes), which includes several important agricultural plant species such as alfalfa, clover, peas, beans and lentils. Until US production began in the late 1940’s and early 1950’s, soybean production occurred solely in China, Indonesia, Japan and Korea (Hymowitz 1970). By the late 1960’s, 25 countries were engaged in soybean production. Most species of Fabaceae, including soybeans, form a symbiotic partnership with the soil bacteria, *Rhizobia*, which results in the formation of nitrogen-fixing root nodule formation (Schubert 1986, Mylona et al. 1995). With recent concern about plant nutrient supplies, specifically N and P, plants in this family contribute immensely to sustainable food production (Vance 2001). Due to its high protein and oil content (Young et al. 1979), many products intended for human consumption today contain soy.

Almost 2 million ha of soybeans are grown in Canada each year with almost 1 million ha grown in Ontario alone (Ontario Ministry of Agriculture Food and Rural Affairs 2011b, Statistics Canada 2011b). Soybean production generated over $1 million CAD in 2011 for Ontario producers (Statistics Canada 2011c), whereas US producers generated nearly $37 billion USD in 2011 (United States Department of Agriculture 2011). Through breeding programs, varieties are developed for specific traits, such as specific oil and protein content, resistance to pests and herbicide tolerance (Ontario Oil & Protein Seed Crop Committee 2010). Although quality is important to all growers, producers of identity-preserved (IP) food grade and soybean seed must pay particular attention to soybean quality because there are specific regulations driven by
consumers and the export market (P. Cornwell, D. Buttenham, personal communications³).

1.7.2 Economic Impact of Feeding Damage

Due to the importance of soybeans as an agricultural crop in North America, assessing and quantifying the economic impact of *C. trifurcata* is crucial. When soybeans are colonized by high populations of *C. trifurcata* during early seedling stages, entire stands can be destroyed (Isely 1930). Although, it is rare for an entire crop to be lost, losses due to adult feeding can be economically damaging to soybean growers. Harmful effects of larval feeding on soybean roots and nodules include up to 50% reductions in yield, plant growth and nitrogen fixation (Newsom et al. 1978), especially in fields with poor soil (McConnell 1915, Leonard and Turner 1918). Moreover, overall plant health is promoted by reducing plant stresses, which includes larval feeding by *C. trifurcata*.

Soybean plants have a great ability to compensate for extra space (Probst 1945), as may be the case when part of the stand is lost to insect feeding, or the loss of leaf tissue via defoliation (Higley 1992, Haile et al. 1998). For example, when adequate moisture was available, soybean plants produced extra leaves and delayed senescence after experiencing up to 66% defoliation (Haile et al. 1998) during the soybean flowering stage (R2) (Fehr et al. 1971). Reproductive stages of soybeans have been defined as flowering (R1-2), pod development (R3-4), seed development (R5-6) and seed maturation (R7-8) (Fehr et al. 1971, Ontario Ministry of Agriculture Food and Rural Affairs 2011a). Defoliation during reproductive stages has more potential to reduce yield compared with defoliation during vegetative stages (Teigen and Vorst 1975). Since pods are the harvestable portion of the crop, the effects of pod-feeding should be carefully considered. Late-season pod-feeding by *C. trifurcata* can impact both yield and quality

---

³ P. Cornwell, Field Marketer/Seed Manager, Hensall District Coop, Hensall, ON, Canada; D. Buttenham, Secretary-Manager, Canadian Soybean Exporters’ Association, Guelph, ON, Canada
up to 50% (Kogan and Herzog 1980, Smelser and Pedigo 1992c). In Iowa, 3.06 kg per ha yield loss was incurred from one beetle per square meter when cages were infested during R6 stage soybeans (Smelser and Pedigo 1992c). Considering high *C. trifurcata* populations have been known to occur during early reproductive stages of soybeans, the impact of feeding during various pod-formation stages should be evaluated. Critical injury levels may differ depending on growth stage as the ability of a soybean plant to compensate for yield reductions declines throughout its development (Smelser and Pedigo 1992c).

Poor quality seed can result in penalties to Canadian farmers at the time of sale (Canadian Grain Commission 2010). Seed quality issues that arise from pathogen infection as a result of pod-feeding also can cause reduced germination (Obopile and Hammond 2001). Reduction in seed quality as a result of *C. trifurcata* pod-feeding can be further exacerbated by delays in harvest (Obopile and Hammond 2001). Although quality is an important consideration, significant yield reductions occurred before economic seed damage in the IP variety Corsoy 79, in which 69% damage of pods was required for economic seed damage to occur (Smelser and Pedigo 1992c). Soybeans may be downgraded based on the presence of slightly stained or mottled seed, heated or mouldy seed, or damaged seed. The Canadian Grain Commission (2010) establishes tolerances for each of these grading factors within its published grade standards (i.e. 0.2% heated or mouldy seed, or 3% damaged seed). Buyer specifications for IP and food grade soybeans demand the highest of quality and there is little to no tolerance for damaged grain (P. Cornwell, D. Buttenham, personal communications⁴).

### 1.7.3 Economic Impact of Pathogens

Losses to yield and quality can occur as a result of secondary damage caused by fungal

---

⁴ P. Cornwell, Field Marketer/Seed Manager, Hensall District Coop, Hensall, ON, Canada; D. Buttenham, Secretary-Manager, Canadian Soybean Exporters’ Association, Guelph, ON, Canada
and viral pathogens on damaged pods (Kogan and Herzog 1980, Shortt et al. 1982). Early infection often results in greater reductions in pod-formation and yield than later infection (Hopkins and Mueller 1984). Significant reductions in yield from BPMV infections have resulted in 52% yield loss (Horn et al. 1973, Hopkins and Mueller 1984), and simultaneous infections with SMV increased yield and quality losses to as much as 80% (Ross 1968, 1969, Tu et al. 1970, Giesler et al. 2002, Hobbs et al. 2003). An economic threshold for BPMV was reached when 40% of plants in the crop were infected, and subsequent control was recommended (Horn et al. 1973).

1.7.4 Economic Injury Level

In the development of integrated pest management (IPM) programs, it is important to explore combinations of management tactics that will have long term effects on the reduction of pest populations with limited impacts on commercial crops and the environment (Pedigo and Rice 2009). To develop appropriate management recommendations, efforts have been made to quantify insect densities and injury caused by insects which would require action by growers to suppress populations. The economic injury level (EIL) is a useful measure of the point where economic damage equals management costs and is determined by measuring crop susceptibility to injury, degree of injury per insect, management costs, and crop value (Pedigo and Rice 2009). Control of an economically damaging pest can be guided through the use of economic thresholds (ET) to make appropriate pest management decisions. An ET, which is the point at which management action should be taken, is calculated as a percent of an economic injury level (EIL) and is frequently set at 80% (Pedigo and Rice 2009).

Inconsistent information used to calculate EIL’s may lead to confusion and inaccurate results. One study found that adult *C. trifurcata* feed for 21 days and consume 0.38 cm² per d
(Smelser and Pedigo 1992a), however, 40 days of feeding at 1.0 cm² per d has been used to calculate EILs (Hunt et al. 1995). Calculations for EILs typically include a range of pest management costs and market values. In Iowa, EILs calculated for *C. trifurcata* using pest management costs of $17.30-25.40 per ha and soybean market values of $0.19-0.38 per kg indicate that EILs range from 14.9 to 42.5 beetles per m² or 2.2 to 6.2 beetles per sweep (assuming 0.76 m row spacing and 25 plants per meter) (Smelser and Pedigo 1992c). A Probabilistic EIL (PEIL) includes uncertainties such as the market values in the EIL calculations, and can be used to make decisions based on comfort levels of risk within an IPM program. The 0-100 ranked percentiles for *C. trifurcata* PEIL’s at early soybean vegetative stages (V1-V2), ranged from 3.88-51.86 beetles per plant; the median PEIL value for *C. trifurcata* in Nebraska was 9.22 beetles per plant compared to an EIL of 9.43 beetles per plant (Peterson and Hunt 2003). It has been proposed that for soybean seedlings, ETs should be the same as EILs because adults have constant consumption rates, but populations are declining early in the season due to mortality of the colonizing generation (Hunt et al. 1995). According to calculations from this study, economic damage can occur with ≥52 beetles per m of row up to V1 seedling stage while ≥127 beetles per m of row after V2 stage are necessary for economic damage to occur (Hunt et al. 1995).

Discrepancies amongst data in consumption and other values used for calculations can cause substantial differences in EILs and economic thresholds. Current recommended ET’s range from ≥13 beetles per m of row to ≥16 beetles per foot of row (Hunt et al. 1995, Ontario Ministry of Agriculture Food and Rural Affairs 2011b). Threshold recommendations which have been made based on the number of beetles per plant, per metre, or per square metre, may potentially be misinterpreted or misunderstood by growers. It is necessary to develop a threshold
based on units that are easy to translate directly from a monitoring program. Depending on the size and stage of the soybean plants, a threshold using number of beetles per plant, per metre of row, or per sweep, may be the most appropriate and transferable.

Forecasting the density of a given population, and subsequent damage, can be useful in an IPM program. Results from a 10 year study demonstrated that abundance of F₂ generations was twice the F₂ density from the year prior or four times the F₁ density of that year (Lam et al., 2001). Combining population forecasts with appropriate ET recommendations can better prepare growers for effective decision making. Currently, thresholds in Ontario for *C. trifurcata* assume two generations per growing season and are based on US recommendations (Ontario Ministry of Agriculture Food and Rural Affairs 2011b). Knowledge of the life cycle and biology of *C. trifurcata* in Ontario is essential for determining the appropriate ET and management recommendations.

1.8 Management

1.8.1 Monitoring

Management strategies for *C. trifurcata* have not been validated in Ontario, but studies in the US have evaluated the efficacy of several management tactics. Employment of any pest management program begins with effective monitoring of pests at critical periods when crops are most susceptible. A variety of techniques have been used to estimate populations present in the crop at a given time. Sticky-board trap catches were less effective at detecting beetle flight than Malaise traps, but they did show directionality of flights (Jeffords et al. 1983). *In situ* counts during early vegetative soybean stages (Fehr et al. 1971) and sweep-net sampling have been commonly used to monitor adults in soybean (Boiteau et al. 1979a, Kogan and Herzog 1980).
When sampling during soybean reproductive stages, beetle counts by sweep net provide more accurate estimates of pod injury than pod sampling (Smelser and Pedigo 1992c). In addition, the sample timing appears to be more important than the sample technique, and sampling between 0800-1400 h or 1400-2000 h provides the best estimates of actual populations (Boiteau et al. 1979a).

1.8.2 Cultural Management

Disrupting synchronization between *C. trifurcata* and their preferred food source by late planting of soybeans has been recommended widely (McConnell 1915, Leonard and Turner 1918, Isely 1930, Witkowski and Echtenkamp 1996), and has reduced the abundance of overwintering adults early in the season and feeding during pod formation by up to 67.9% (Pedigo and Zeiss 1996, Witkowski and Echtenkamp 1996). In Iowa, a 14 d delay in planting was sufficient to reduce colonizing populations (Zeiss and Pedigo 1996), and although significant differences in yield were not observed, economic damage did not occur indicating potential effectiveness of this approach as a management tactic (Pedigo and Zeiss 1996). Late planting was suggested to limit reproductive and injury potential of F₁ beetles in areas where there may only be a partial F₂ generation (Hammack et al. 2010). Some risks are associated with delayed planting in terms of unpredictable, adverse spring weather events, and unforeseen consequences can occur when a single management tactic is widely adopted. However, no evidence exists to suggest that large-scale employment of this tactic would be harmful to soybean growers (Zeiss and Pedigo 1996). Changing weather and warmer springs may result in a growing trend toward planting soybean earlier, which may effectively synchronize *C. trifurcata* colonization depending on locality and voltinism.

Placement of soybean crops according to landscape suitability should be considered to
minimize the availability of other food sources of the beetles and overwintering sites (Zeiss and Pedigo 1996). Removal of wild legumes may be appropriate in some situations (Chittenden 1897). Crop rotation, minimal use of nitrogen fertilizer, and chisel-tillage have been shown to suppress *C. trifurcata* populations, with fewer beetles found in plots treated with low N and chisel-tilled by comparison with ridge-tilled plots (Leonard and Turner 1918, Hammack et al. 2010).

### 1.8.3 Chemical Management

Chemical options for Ontario soybean growers for treatment of *C. trifurcata* currently include insecticidal seed treatments (thiamethoxam) and foliar applications (i.e. lambda-cyhalothrin, dimethoate, and imidaclloprid + deltamethrin) (Ontario Ministry of Agriculture Food and Rural Affairs 2011-2012). Thiamethoxam, a second-generation neonicotinoid (Maienfisch et al. 2001), is an effective seed treatment used to control *C. trifurcata* during early soybean vegetative stages (Witkowski and Echtenkamp 1996, Piitz 2012). Neonicotinoid seed treatments (Bradshaw et al. 2008) or in-furrow applications of carbamate, a systemic soil insecticide, at planting (Witkowski and Echtenkamp 1996) can reduce overwintered populations of *C. trifurcata*. Insecticide-based management programs targeting *F₀* and *F₁* populations can, under high beetle pressure, improve yield and quality (Krell et al. 2004, Bradshaw et al. 2008). The vast majority of soybean in Ontario is planted with thiamethoxam seed treatments. Although there are few cases of resistance to neonicotinoids, several insects have developed resistance to this chemical class (Nauen and Denholm 2005).

When thresholds are reached later in the season, after seed treatments are no longer effective, foliar applications can be used to control *C. trifurcata*. A growing trend away from organophosphates (dimethoate) has led to an increase in use of other efficacious chemical classes
including pyrethroids (lambda-cyhalothrin) (Vijverberg et al. 1982). Although insecticides are necessary during population outbreaks, relying solely on insecticides for control may result in development of insecticidal resistance. In the Mississippi Delta, *C. trifurcata* tolerance to pyrethroid insecticides has been documented (Musser et al. 2012).

### 1.8.4 Host Plant Resistance

Differences in yield loss and significant differences in EIL’s can occur among soybean cultivars. Yield losses from larvae of another defoliating insect, green cloverworm (*Hypena scabra* Fabricus), ranged from 15-70% and EIL’s ranged from 21-46 larvae per m of row among cultivars when using artificial defoliation techniques and daily consumption rates of larvae (Haile et al. 1998). Differences among cultivars were categorized by maturity group, leaf area, growth habit and morphology, all of which contribute to the impact of defoliation on yield (Haile et al. 1998). Resistance to *C. trifurcata* feeding has been documented in some cultivars that have densely pubescent pods (Shortt et al. 1982, Lam and Pedigo 2001). Late-maturing cultivars may experience more pod-feeding and subsequent germination loss (Shortt et al. 1982).

### 1.8.5 Biological Control

Several parasitoids of *C. trifurcata* have been observed in the field and reared in laboratories, such as the internal parasite *Celatoria diabroticae* (Shimer) (Diptera: Tachinidae) (McConnell 1915, Eddy and Nettles 1930, Isely 1930) and *Hyalomyodes triangulifer* (Loew) (Diptera: Tachinidae). However, control of beetles has been limited with a maximum parasitism rate of 7% observed in the field (Marrone et al. 1983). The parasitoid, *Medina* n. sp. (Diptera: Tachinidae), which overwinters as a larva in the diapausing beetle, was identified in 1983 in Minnesota and 40% parasitism rates were observed in soybean crops in close proximity to alfalfa crops (Loughran and Ragsdale 1986a).
1.8.6 Pathogen and Vector Management

One method used to control insect-vectored pathogens is to control the insect vectors. Reduction in BPMV infection can occur when seed treatments are used to control overwintered populations of *C. trifurcata* (Bradshaw et al. 2008). Lambda-cyhalothrin insecticide seed treatments, targeted at the F₀ and F₁ generations of *C. trifurcata*, potentially reduce beetle abundance, BPMV infection in the field by 32%, and seed coat mottling by 31% (Krell et al. 2004). With these treatments, yields were 525 kg per ha (7.8 bu per ac) higher than in untreated control plots (Krell et al. 2004).

Two pests that occur simultaneously in a crop and vector similar viruses with potential additive effects warrant further examination. Efforts to harmonize controls of *C. trifurcata* with those for *A. glycines* have not been successful thus far. Seed treatments and early season foliar insecticides used for managing *C. trifurcata* did not provide sufficient control of *A. glycines*, which reached economically damaging populations later in the season (Johnson et al. 2008). Yet potential exists for monitoring and management of the two pests during high populations of *A. glycines* and active pod-feeding by *C. trifurcata* in August (Smelser and Pedigo 1992b). Delayed planting was evaluated for management of *C. trifurcata* as well as for the impact on BPMV incidence (Krell et al. 2005). However, results were inconsistent and this practice is not considered an effective management tactic for this disease (Krell et al. 2005).

Currently no BPMV-resistant soybean varieties are commercially available. Susceptibility to viral and fungal pathogen infections may differ among cultivars (Hobbs et al. 2003, Zheng et al. 2005b), but most varieties tested acquire BPMV (Schwenk and Nickell 1980). *Glycine, Phaseolus,* and *Vigna* species were screened for resistance and although differences in aggressiveness of BPMV were apparent among species, no evidence of resistance was found in
soybean (Mozzoni et al. 2009). Conversely, transgenic soybean lines have shown potential resistance to BPMV (Reddy et al. 2001) and *Glycine tomentella* Hayata has provided evidence of the presence of resistance traits within the *Glycine* genus (Zheng et al. 2005b), with potential for integration into soybean cultivars in the future.

### 1.9 Research Objectives

Although research has been conducted throughout the US, increased knowledge of the biology of *C. trifurcata* in southwestern Ontario is necessary to evaluate the potential impact of this pest. Increasing BPMV infection and potential additive effects when combined with SMV as seen in the US have the potential to occur in southwestern Ontario.

Therefore, the objectives of this thesis were to 1.) examine the phenology, life cycle and degree day requirements of *C. trifurcata* in Ontario; 2.) evaluate the impact of late-season pod-feeding on soybean yield and quality; 3.) assess the extent of virus transmission; 4.) develop an economic threshold for late-season pod-feeding; and 5.) develop appropriate management recommendations in the context of the current agronomic practices Ontario soybean producers.
CHAPTER TWO

Temperature-Dependent Development and Degree Day Requirements of Bean Leaf Beetle, *Cerotoma trifurcata*, under Constant Temperature Regimes.

2.1 Abstract

Temperature-dependent development of bean leaf beetle, *Cerotoma trifurcata* (Forster), was evaluated in the laboratory under five constant temperature regimes: 15.0, 18.8, 23.4, 26.1, and 30.9°C. Linear and nonlinear models were used to compare developmental rates for life stage events using number of days to eclosion, pupation, adult emergence and egg deposit to adult emergence. Linear models provided a better fit than nonlinear models for all life stage events according to Akaike information criterion (AIC) values. The developmental rates were significantly higher at 30.9°C than at all other temperature regimes for all life stage events. Thermal constants were calculated from the linear model and were used to determine degree day requirements to complete each life stage event. Degree day (DD) requirements to complete development for eclosion, pupation, adult emergence and from egg deposition to adult emergence were 148 ± 3.4 (base 10.25°C), 348 ± 22.7 (base 9.25°C), 122 ± 19.7 (base 9.47°C), and 628 ± 43.2 (base 9.31°C) DD, respectively. The best estimate of lower developmental threshold for *C. trifurcata* from this study was 10.25 ± 0.381°C.

**Key Words**  Bean leaf beetle, *Cerotoma trifurcata*, predictive model, poikilotherm development, thermal requirements
2.2 Introduction

The bean leaf beetle, *Cerotoma trifurcata* (Forster), is an indigenous and economically damaging pest of soybeans, *Glycine max* (L.) Merr. (Fabaceae) in the US (Chittenden 1897). While historical documents report its occurrence in southern Canada as a migratory late-season pest (Chittenden 1897, Isely 1930), recent evidence suggests that it is now overwintering, and has become of greater concern to Ontario growers (personal communication, T. Baute, A. Schaaafsma, and J. Smith, 2009). Its life history and development in this northern region of distribution is undetermined and this information is crucial to the development of an appropriate management strategy.

Poikilothermic models have been used to accurately describe temperature-dependent development of insects (Campbell et al. 1974, Wagner et al. 1984a, Wagner et al. 1984b, Wagner et al. 1991). Eclosion of a close relative of *C. trifurcata*, the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), was modeled using developmental rates at a range of temperatures in the laboratory (Schaafsma et al. 1991). Thermal models for development have been examined for the soybean aphid (*Aphis glycines* Matsumura) and the aphids’ primary host, common buckthorn (*Rhamnus cathartica* L.) (Bahlai et al. 2010), as well as a soybean aphid parasitoid, *Aphelinus certus* Yasnosh (Frewin et al. 2010). Ultimately, insect development takes a nonlinear form, where minimum, optimum and maximum thresholds can be determined from nonlinear curve fitting (Briere et al. 1999). Yet, there is a linear portion of development, which is often a sufficient representation of growth rates (Campbell et al. 1974). Thermal constants are heat accumulations, or degree days, that are required for a species to complete development (Damos and Savopoulou-Soultani 2012). Thermal requirements for development can be modeled to predict insect phenology in the field (Schaafsma et al. 1991, Legg et al. 2000).
Degree day requirements for development of *C. trifurcata* were studied in the US (Zeiss et al. 1996, Hammack et al. 2010) but some evidence suggests that developmental times and minimum developmental thresholds may be influenced by cooler temperatures or shorter seasons as insects adapt to northern climates (Honék 1996). For example: an Illinois population of western corn rootworm took longer to develop than populations studied in Minnesota and South Dakota (Levine et al. 1992). Conversely, the golden-eyed lacewing, *Chrysopa oculata* Say, has consistent thresholds over its geographic range (Tauber et al. 1987). Degree day models and minimum thresholds for *C. trifurcata* development reported in the literature are not consistent (Zeiss et al. 1996, Hammack et al. 2010). Because *C. trifurcata* development and voltinism is uncertain in this new northern region of range expansion, it is important to have knowledge of these characteristics when developing management programs.

The objectives of this study were: 1) to determine the effect of temperature on the development of *C. trifurcata* in southwestern Ontario; and 2) to establish degree day models for development through life stages, which could be used as a predictive tool in the field.

### 2.3 Materials and Methods

To evaluate development at constant temperatures, *C. trifurcata* were reared according to Zeiss et al. (1996) in growth chambers (Conviron, Model E7H, Winnipeg, Manitoba, Canada) using 14L:10D photoperiod and target temperature regimes of 15, 19, 23, 27, and 31°C. Adults were held at 25°C. Experimental units were individual eggs that were randomly assigned to a temperature regime. Each individual was assessed for time to hatch, pupation and adult emergence. Temperature and relative humidity were recorded in each growth chamber using data loggers (Onset Hobo® Model U23-001 Pro V2, Cape Cod, Massachusetts).
Temperatures within growth chambers deviated from desired temperatures and were evaluated to determine whether average recorded temperatures were appropriate representations of desired temperatures by comparing actual degree day (DD$_A$) to predicted degree day (DD$_P$) accumulations (Table 2.1). Actual number of days and degree day accumulations were determined using a minimum developmental threshold of 10.25°C as determined by this study (see Results) for life stage events of *C. trifurcata* (eclosion, pupation, adult emergence, egg deposit to adult emergence). Daily DD$_P$ were computed using average temperatures throughout the 7 month period for each regime minus the minimum developmental threshold (base 10.25°C). DD$_P$ accumulations were computed using the actual number of days to the event multiplied by the daily DD$_P$. Regression analysis was performed on DD$_P$ against DD$_A$ accumulations to determine if there were differences between the two values. The intercept from each regression was divided by the daily DD$_P$ to determine whether the observed and predicted number of days differed (Table 2.1). All slopes from regressions approached 1, with values <1 representing an over-fitted model (Steyerberg et al. 2010). Intercepts were largest for egg deposit to adult emergence in the 19, 23 and 27°C regimes. Most predictions were less than a day different from observed values based on the number of days ($a$/DD$_P$). However, predictions were nearly 10, 5 and 6 d longer for egg deposit to adult emergence at 19, 23 and 27°C. Consideration should be given to the long term effects of the temperature fluctuations when evaluating the degree day accumulations in this study; however, regression analysis indicated that average temperature over the 7 month period was an accurate reflection of desired temperature. Only significant relationships (P < 0.05) are presented in the results. Growth chamber conditions are presented in Table 2.1.
Table 2.1. Growth chamber temperature conditions for *C. trifurcata* rearing at University of Guelph, Ontario, including coefficients for regressions of predicted\(^1\) against observed\(^2\) degree day (DD) accumulations using a minimum developmental threshold of 10.25°C.

<table>
<thead>
<tr>
<th>Chamber no.</th>
<th>Desired temperature (°C)</th>
<th>Mean temperature ± SD (°C)</th>
<th>Predicted Daily DD (DD(_P)) (Base 10.25°C)</th>
<th>Life Stage</th>
<th>Intercept (a)</th>
<th>Slope (b)</th>
<th>(R^2)</th>
<th>No. days ((a/DD))^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>15.0 ± 1.31</td>
<td>4.75</td>
<td>Eclosion</td>
<td>8.62</td>
<td>0.979</td>
<td>0.97</td>
<td>1.81</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>18.8 ± 0.55</td>
<td>8.59</td>
<td>Eclosion</td>
<td>-1.48</td>
<td>1.004</td>
<td>0.99</td>
<td>-0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pupation</td>
<td>19.26</td>
<td>0.933</td>
<td>0.99</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adult Emergence</td>
<td>0.02</td>
<td>0.967</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Egg Deposit to Adult Emergence</td>
<td>84.93</td>
<td>0.859</td>
<td>0.86</td>
<td>9.89</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>23.4 ± 0.95</td>
<td>13.2</td>
<td>Eclosion</td>
<td>4.21</td>
<td>0.964</td>
<td>0.98</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pupation</td>
<td>10.35</td>
<td>0.958</td>
<td>0.99</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adult Emergence</td>
<td>2.76</td>
<td>0.959</td>
<td>0.98</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Egg Deposit to Adult Emergence</td>
<td>64.04</td>
<td>0.891</td>
<td>0.99</td>
<td>4.85</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>26.1 ± 1.56</td>
<td>15.88</td>
<td>Eclosion</td>
<td>14.10</td>
<td>0.859</td>
<td>0.95</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pupation</td>
<td>17.13</td>
<td>0.908</td>
<td>0.97</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adult Emergence</td>
<td>10.72</td>
<td>0.919</td>
<td>0.98</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Egg Deposit to Adult Emergence</td>
<td>99.00</td>
<td>0.806</td>
<td>0.98</td>
<td>6.23</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>30.9 ± 1.29</td>
<td>20.65</td>
<td>Eclosion</td>
<td>1.50</td>
<td>1.006</td>
<td>1.00</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pupation</td>
<td>-9.33</td>
<td>1.043</td>
<td>0.99</td>
<td>-0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adult Emergence</td>
<td>2.60</td>
<td>1.001</td>
<td>1.00</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Egg Deposit to Adult Emergence</td>
<td>8.39</td>
<td>1.015</td>
<td>0.98</td>
<td>0.41</td>
</tr>
</tbody>
</table>

\(^1\)Predicted degree day accumulations were calculated using average temperature over the 7 month study, minus minimum developmental threshold, multiplied by the number of days to the event. 
\(^2\)Observed degree day accumulations were calculated using average daily temperatures minus the minimum developmental threshold accumulated for each day to the event. 
\(^3\)Number of days difference between predicted and observed DD accumulations was calculated using the intercept from the regression divided by the corresponding predicted daily degree days.
2.3.1 Rearing Methods

*C. trifurcata* were reared according to modified methods from Zeiss et al. (1996). Adults were collected from overwintering sites around the perimeter of former soybean fields in early spring and soybean fields through the growing season near Ridgetown, ON, between 15 February and 15 September, 2011. Mating pairs of adults were placed in 500 mL deli cups (Solo® Cup Company, Highland Park, Illinois, US) with a thin layer of 1% agar on the bottom to maintain moisture. The agar was covered with a sterilized filter paper (Fisherbrand® P8, 9 cm, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Adults were provided *ad libitum* with a soybean trifoliolate held in a floral water pick (Syndicate Sales, Inc., Kokomo, Indiana, US). Genetic diversity in mating pairs was assured by collecting and introducing new wild adults throughout the study period. Individual eggs were transferred onto sterile filter paper (Whatman™ No. 1, 3.2 cm, Whatman Ltd., Clifton, New Jersey, USA) with a camel’s hair brush, held in 30 mL polystyrene cups (Solo® Cup Company, P100, Lake Forest, Illinois) with a thin layer of 2% agar solution. Egg containers were randomly assigned to the five temperature regimes.

Complete larval emergence was the indicator for egg eclosion. Newly eclosed larvae were transferred individually to the concave side of a cowpea cotyledon, which were refreshed as needed. The development of larval instars could not be determined because larvae burrowed into cotyledons and mortality due to manipulation was high. Larvae not emerging from cotyledons after ~ 8 weeks were assumed to have died and were discarded. As third instars matured into prepupae, they burrowed into the agar down the side of the portion cup. Pupae were transferred to a new portion cup containing lightly-moistened, fine, granulated, washed play sand (All Treat Farms Limited, Arthur, Ontario, Canada) and gently covered with a thin layer of sand. Teneral
adults emerged from the sand. Adults that emerged were sexed and placed in mating pairs in the adult growth chamber; however, few eggs were recovered from these containers. All containers were checked daily from mid-February to mid-October until adults ceased laying viable eggs.

2.3.2 Statistical Analysis

Overall rates of survivorship were determined based on numbers of individuals that survived through life stage events for all temperature regimes; however, data from the 15°C regime were excluded from the remaining analyses due to low rates of eclosion and no larval survivorship. To satisfy assumptions of normality and achieve linearity in regression analyses, data were transformed based on the highest Shapiro-Wilk statistic. Outliers were identified using Lund’s test of studentized residuals (Bowley 2008, Rotondi and Koval 2009). Mean temperature (Table 2.1) over 7 months of rearing was used in all analyses.

All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Linear and nonlinear regressions were performed using PROC GLM and PROC NLIN, respectively, on developmental rates (day\(^{-1}\)) versus mean temperature (°C) for life stage events of C. trifurcata (eclosion, pupation, adult emergence and egg deposit to adult emergence). Linear models took the form:

\[ R(T) = a + bT \]  

where \( R(T) \) is the developmental rate at temperature \( T \) (°C), \( a \) is the Y-intercept and \( b \) is the slope (Campbell et al. 1974). The minimum developmental threshold was estimated for each life stage using the X-intercept and took the form:
Minimum developmental thresholds were compared using the ANCOVA procedure in PROC MIXED (Shi et al. 2010).

The following nonlinear temperature-dependent growth model (Briere et al. 1999) was chosen because it estimated the minimum, optimal and upper thresholds with the fewest parameters:

\[
X_{\text{INT}} = -\frac{a}{b}
\]  

[2]

where \( R(T) \) is the developmental rate at temperature \( T \) (°C), \( T_0 \) is an estimate of the upper developmental threshold, \( T_L \) is an estimate of the minimum developmental threshold, and \( \alpha \) is a constant (Briere et al. 1999). Fitted curves were obtained using a nonlinear iterative regression based on the Marquardt method. Optimal developmental rates, \( T_{OPT} \), were estimated using equation 4 (Briere et al. 1999):

\[
T_{OPT} = \frac{4T_L + 3T_0 + \sqrt{16T_L^2 + 9T_0^2 - 16T_0T_L}}{10}
\]

[4]

2.3.3 Degree Day Model

Degree days were calculated in 30 min. increments (base 11.58°C) (Zeiss et al. 1996) for
each life stage event under each temperature regime. With determination of a more accurate lower developmental threshold (base 10.25°C) from this study (see Results), degree day requirements were recalculated for each life stage. These data were subjected to ANOVA using PROC MIXED. Degree day requirements to complete each life stage event were also estimated from the slope of linear regressions (DD = $b^{-1}$) (Campbell et al. 1974).

2.4 Results

2.4.1 Survivorship

Survivorship from egg to adult was low over all temperature regimes (Table 2.2). Greater than 50% hatch was observed in all temperature regimes except 15.0°C. Percent survivorship at pupal and adult stages was highest in the 23.4 and 26.1°C regimes. The ratio of male to female adult emergence was nearly equal at 23.4 and 26.1°C (Table 2.3). However, a male-biased sex ratio was observed at 18.8 and 30.9°C. Temperatures of 23-26°C appear to be optimal for C. trifurcata.

2.4.2 Developmental Rate

The number of days to complete each life stage decreased with increasing temperature (Table 2.4) and developmental rates increased linearly with temperature for all life stages (Fig. 2.1). According to Akaike information criterion (AIC) values (Burnham and Anderson 2002, Burnham et al. 2011) (Table 2.5), linear models provided a better fit for C. trifurcata developmental rates than nonlinear models for eclosion, pupation, adult emergence, and egg deposit to adult emergence. Parameter estimates for linear and nonlinear models are provided in Table 2.5.

Optimal developmental rates for eclosion, pupation, adult emergence, and from egg deposit
Table 2.2. Percentage of survivorship for each life stage event of *C. trifurcata* individuals reared in environmentally-controlled growth chambers under five constant temperatures at the University of Guelph, Guelph, Ontario, Canada.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Total no. eggs</th>
<th>Eclosion</th>
<th>Pupa</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>% stage</td>
</tr>
<tr>
<td>15.0</td>
<td>149</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18.8</td>
<td>500</td>
<td>302</td>
<td>43</td>
<td>14</td>
</tr>
<tr>
<td>23.4</td>
<td>558</td>
<td>293</td>
<td>82</td>
<td>28</td>
</tr>
<tr>
<td>26.1</td>
<td>513</td>
<td>301</td>
<td>82</td>
<td>27</td>
</tr>
<tr>
<td>30.9</td>
<td>576</td>
<td>301</td>
<td>21</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 2.3. Ratio of male:female adult *C. trifurcata* emergence from individuals reared in environmentally-controlled growth chambers under four constant temperatures at the University of Guelph, Guelph, Ontario, Canada.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Number of male</th>
<th>Number of female</th>
<th>Ratio M:F</th>
<th>Number unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.8</td>
<td>3</td>
<td>1</td>
<td>3:1</td>
<td>3</td>
</tr>
<tr>
<td>23.4</td>
<td>21</td>
<td>18</td>
<td>1.2:1</td>
<td>5</td>
</tr>
<tr>
<td>26.1</td>
<td>19</td>
<td>15</td>
<td>1.3:1</td>
<td>2</td>
</tr>
<tr>
<td>30.9</td>
<td>5</td>
<td>1</td>
<td>5:1</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 2.4. Mean developmental days and rates (±SE) for life stage events of *C. trifurcata* reared in environmentally-controlled growth chambers under four constant temperature regimes at the University of Guelph, Guelph, Ontario, Canada.

<table>
<thead>
<tr>
<th>Life Stage</th>
<th>Temperature (°C)</th>
<th>N</th>
<th>No. days</th>
<th>Rate (d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Egg hatch</strong></td>
<td>18.8</td>
<td>297</td>
<td>18</td>
<td>0.056 ± 0.0013 d</td>
</tr>
<tr>
<td></td>
<td>23.4</td>
<td>286</td>
<td>11</td>
<td>0.092 ± 0.0014 c</td>
</tr>
<tr>
<td></td>
<td>26.1</td>
<td>291</td>
<td>9</td>
<td>0.110 ± 0.0014 b</td>
</tr>
<tr>
<td></td>
<td>30.9</td>
<td>277</td>
<td>7</td>
<td>0.138 ± 0.0014 a</td>
</tr>
<tr>
<td><strong>Pupation</strong></td>
<td>18.8</td>
<td>36</td>
<td>38</td>
<td>0.027 ± 0.0014 d</td>
</tr>
<tr>
<td></td>
<td>23.4</td>
<td>74</td>
<td>25</td>
<td>0.041 ± 0.0010 c</td>
</tr>
<tr>
<td></td>
<td>26.1</td>
<td>77</td>
<td>20</td>
<td>0.050 ± 0.0010 b</td>
</tr>
<tr>
<td></td>
<td>30.9</td>
<td>18</td>
<td>17</td>
<td>0.059 ± 0.0020 a</td>
</tr>
<tr>
<td><strong>Adult emergence</strong></td>
<td>18.8</td>
<td>16</td>
<td>3</td>
<td>0.064 ± 0.0177 d</td>
</tr>
<tr>
<td></td>
<td>23.4</td>
<td>9</td>
<td>39</td>
<td>0.116 ± 0.0049 c</td>
</tr>
<tr>
<td></td>
<td>26.1</td>
<td>7</td>
<td>31</td>
<td>0.138 ± 0.0055 b</td>
</tr>
<tr>
<td></td>
<td>30.9</td>
<td>6</td>
<td>8</td>
<td>0.172 ± 0.0108 a</td>
</tr>
<tr>
<td><strong>Egg deposit to adult emergence</strong></td>
<td>18.8</td>
<td>7</td>
<td>68</td>
<td>0.015 ± 0.0011 d</td>
</tr>
<tr>
<td></td>
<td>23.4</td>
<td>43</td>
<td>45</td>
<td>0.022 ± 0.0005 c</td>
</tr>
<tr>
<td></td>
<td>26.1</td>
<td>36</td>
<td>37</td>
<td>0.027 ± 0.0005 b</td>
</tr>
<tr>
<td></td>
<td>30.9</td>
<td>10</td>
<td>30</td>
<td>0.034 ± 0.0010 a</td>
</tr>
</tbody>
</table>

LS means within columns within life stage followed by the same letter are not significantly different (Fisher’s Protected LSD, *P > 0.05*).
Figure 2.1. Temperature-dependent development for *Cerotoma trifurcata* reared in environmentally-controlled growth chambers under four constant temperature regimes (18.8, 23.4, 26.1, 30.9°C) at the University of Guelph, Guelph, Ontario, Canada. (A) Eclosion. (B) Pupation. (C) Adult emergence. (D) Egg deposit to adult emergence. Solid line represents the fitted physiological growth curve. Dotted line represents the linear regression. Circles represent means from linear regression.
Table 2.5. Parameter estimates (±SE) of linear and nonlinear regressions for *C. trifurcata* developmental life stages under four temperatures in environmentally-controlled growth chambers at the University of Guelph, Guelph, Ontario, Canada.

**Linear Model**

<table>
<thead>
<tr>
<th>Life Stage</th>
<th>Slope (b) (x10^3)</th>
<th>Y-intercept (a)</th>
<th>X-intercept</th>
<th>n</th>
<th>AIC</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eclosion</td>
<td>6.77 ± 0.157</td>
<td>-0.0694 ± 0.00395</td>
<td>10.25 ± 0.381</td>
<td>1151</td>
<td>-5356.5</td>
<td>0.62</td>
</tr>
<tr>
<td>Pupation</td>
<td>2.87 ± 0.187</td>
<td>-0.0266 ± 0.00459</td>
<td>9.25 ± 1.003</td>
<td>205</td>
<td>-1320.5</td>
<td>0.54</td>
</tr>
<tr>
<td>Adult Emergence</td>
<td>8.21 ± 1.324</td>
<td>-0.0777 ± 0.03332</td>
<td>9.47 ± 4.434</td>
<td>81</td>
<td>-306.1</td>
<td>0.33</td>
</tr>
<tr>
<td>Egg deposit to adult emergence</td>
<td>1.59 ± 0.110</td>
<td>-0.0148 ± 0.00275</td>
<td>9.31 ± 1.090</td>
<td>96</td>
<td>-792.1</td>
<td>0.69</td>
</tr>
</tbody>
</table>

**Nonlinear Model**

<table>
<thead>
<tr>
<th>Life Stage</th>
<th>a (x10^2)</th>
<th>T_L</th>
<th>T_O</th>
<th>n</th>
<th>AIC</th>
<th>T_Opt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eclosion</td>
<td>6.3 ± 0.68</td>
<td>41.2 ± 1.49</td>
<td>8.76 ± 0.963</td>
<td>1151</td>
<td>-3759.2</td>
<td>33.8</td>
</tr>
<tr>
<td>Pupation</td>
<td>2.8 ± 0.64</td>
<td>39.9 ± 2.83</td>
<td>7.98 ± 2.295</td>
<td>205</td>
<td>-840.6</td>
<td>32.8</td>
</tr>
<tr>
<td>Adult Emergence</td>
<td>8.5 ± 5.40</td>
<td>40.0 ± 6.99</td>
<td>9.37 ± 6.249</td>
<td>81</td>
<td>-240.9</td>
<td>33.1</td>
</tr>
<tr>
<td>Egg deposit to adult emergence</td>
<td>1.4 ± 0.44</td>
<td>42.4 ± 4.60</td>
<td>7.18 ± 3.433</td>
<td>96</td>
<td>-479.1</td>
<td>34.7</td>
</tr>
</tbody>
</table>

Linear models include the minimum developmental threshold in °C (X-intercept). Nonlinear models include a constant α, the upper developmental threshold $T_L$, and the minimum developmental threshold $T_O$, and optimal developmental rates were achieved at temperature $T_{OPT}$. For all models, total no. of life stage observations is n. AIC values were calculated from log likelihood estimates and used to compare linear and nonlinear regressions for the same life stage. For all models, $P < 0.0001$. 
to adult emergence were calculated to occur at 33.8, 32.8, 33.1 and 34.7°C, respectively (Table 2.5). These optimal temperatures are slightly above the highest temperature regime (30.9°C) included in this study where developmental rates appeared to be continuing to increase.

Minimum developmental thresholds determined by linear regression were higher than those from nonlinear models for all life stages (Table 2.5). The slope determined by linear regression for days to eclosion was significantly higher than those for the other developmental stages examined \((P < 0.0001, F = 128.66, df = 3)\), which resulted in a higher minimum developmental threshold.

Based on criteria outlined by Campbell et al. (1974) for assessing extrapolated lower developmental thresholds, the minimum developmental threshold estimate of 10.25 ± 0.38°C for eclosion is the most accurate of those determined here.

**2.1.1 Degree Day Model**

Degree day accumulations at 30.9°C were significantly higher than the other temperature regimes for eclosion, pupation and egg deposit to adult emergence, using a minimum developmental threshold of 11.58°C (Table 2.6). Using a minimum developmental threshold of 10.25°C, degree day accumulations at 18.8 and 30.9°C were significantly higher than the other temperature regimes for eclosion and pupation (Table 2.6).

Observed degree day accumulations (base 10.25°C) to 50% eclosion for 18.8, 23.4, 26.1 and 30.9°C were 165, 145, 144 and 148, respectively (Fig. 2.2). Observed degree day accumulations to 50% pupation for 18.8, 23.4, 26.1 and 30.9°C were 341, 319, 316 and 360, respectively (Fig. 2.3). These values are within ~ 4 and 12 degree days of the calculated means for eclosion and pupation, respectively (Table 2.6). Observed degree days (base 10.25°C) to 50% adult emergence for 18.8, 23.4, 26.1 and 30.9°C were 133, 111, 118 and 127, respectively (Fig. 2.4). Observed degree days (base 10.25°C) for 50% egg deposit to adult emergence for 18.8, 23.4,
Table 2.6. Means comparison of degree day models for *C. trifurcata* development through life stage events while reared in growth chambers held at four temperature regimes (°C). Degree day models presented are LS means (±SE) of actual degree day accumulations (base 11.58 and 10.25°C) calculated for 30 min. intervals for each temperature regime and the estimated degree days calculated from the inverse of the slope \((b)\) of the linear regression.

<table>
<thead>
<tr>
<th>Estimate Source</th>
<th>Degree Day Accumulations</th>
<th>Egg Deposit to Adult Emergence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eclosion</td>
<td>Pupation</td>
</tr>
<tr>
<td><strong>Base 11.58°C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.8°C</td>
<td>136 ± 1.8 b</td>
<td>289 ± 9.4 b</td>
</tr>
<tr>
<td>23.4°C</td>
<td>133 ± 1.9 b</td>
<td>298 ± 6.5 b</td>
</tr>
<tr>
<td>26.1°C</td>
<td>132 ± 1.8 b</td>
<td>290 ± 6.4 b</td>
</tr>
<tr>
<td>30.9°C</td>
<td>151 ± 1.9 a</td>
<td>343 ± 13.3 a</td>
</tr>
<tr>
<td><strong>Base 10.25°C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.8°C</td>
<td>161 ± 2.0 a</td>
<td>343 ± 10.5 ab</td>
</tr>
<tr>
<td>23.4°C</td>
<td>148 ± 2.1 b</td>
<td>331 ± 7.3 bc</td>
</tr>
<tr>
<td>26.1°C</td>
<td>145 ± 2.0 b</td>
<td>317 ± 7.2 c</td>
</tr>
<tr>
<td>30.9°C</td>
<td>161 ± 2.1 a</td>
<td>367 ± 14.8 a</td>
</tr>
</tbody>
</table>

| Inverse of the Slope\(^1\) | 148 ± 3.4 | 348 ± 22.7 | 122 ± 19.7 | 628 ± 43.2 |

LS means within columns for each base temperature followed by the same letter are not significantly different (Fisher’s Protected LSD, \(P > 0.05\)).

\(^1\) Degree day estimates from the inverse of the slope of the linear regression were not used in the ANOVA for life stage degree day models. These values represent thermal constants with lower development thresholds (base temperatures) of 10.25, 9.25, 9.47 and 9.31°C, for eclosion, pupation, adult emergence and egg deposit to adult emergence, respectively.
Figure 2.2. Relative frequency histograms and cumulative percent distributions of *C. trifurcata* degree day accumulations to eclosion (base 10.25°C) after rearing in growth chambers under four constant temperature regimes (°C).
Figure 2.3. Relative frequency histograms and cumulative percent distributions of *C. trifurcata* degree day accumulations to pupation (base 10.25°C) after rearing in growth chambers under four constant temperature regimes (°C).
Figure 2.4. Relative frequency histograms and cumulative percent distributions of *C. trifurcata* degree day accumulations to adult emergence (base 10.25°C) after rearing in growth chambers under four constant temperature regimes (°C).
26.1 and 30.9°C were 600, 600, 567 and 636, respectively (n=7, 38, 35 and 10, respectively) (Fig. 2.5). These values were less than 12 and 10 degree days from the calculated means for adult emergence and egg deposit to adult emergence, respectively (Table 2.6).

2.2 Discussion

The nonlinear model from this study can be further evaluated using the thermal window, which is the difference between $T_O$ and $T_L$ (Dixon et al. 2009). Most insects develop within a 20°C maximum to minimum temperature range of (Schoolfield et al. 1981, Dixon et al. 2009). The nonlinear model estimates for maximum to minimum temperature ranges here exceed 30°C for all life stages examined. This result supports the conclusion that the linear model is more suited to the observations made in this study and the parameter estimates from the nonlinear model may not accurately reflect *C. trifurcata* development. Furthermore, the linear portion of insect development most often lies within the temperature range of its natural habitat (Campbell et al. 1974) and thermal soil conditions for *C. trifurcata* in the field were similar to the temperature range of this study.

Previous studies have found insect development to be isomorphic between life stages, where all life stages experience the same developmental rates and have the same minimum thresholds (van Rijn et al. 1995, Jarošík et al. 2004). However, the minimum threshold for *C. trifurcata* eclosion was higher than those for other developmental stages. Differences between minimum thresholds may be a result of a small sample size and uncertainties of fitted parameters (Bergant and Trdan 2006). According to Campbell et al. (1974), extrapolation of the lower threshold may result in a poor estimate and a minimum of 3 or 4 temperatures with 50 specimens each should be used when determining the lower threshold by extrapolation. These requirements were only
Figure 2.5. Relative frequency histograms and cumulative percent distributions of *C. trifurcata* degree day accumulations from egg deposit to adult emergence (base 10.25°C) after rearing in growth chambers under four constant temperature regimes (°C).
satisfied for all four temperature regimes for eclosion and, therefore the lower threshold estimate of 10.25 ± 0.381°C should be the most accurate of those determined here. Bergant and Trdan (2006) evaluated thermal constants from laboratory experiments and recommended at least five temperatures within the linear portion of development. Five temperature regimes were included in this study, however, virtually no development occurred at 15°C. Developmental rates may also have been affected by rearing *C. trifurcata* on cowpea cotyledons rather than on soybean, its usual host (Hughes 1963, Campbell et al. 1974).

Overall, rearing *C. trifurcata* from egg to adult was challenging. Regardless of temperature regime, survivorship was low, but consistent with other reports (Zeiss et al. 1996). Low survivorship from the pupal to adult stage could be a result of handling issues. In this study, substantial differences in adult sex ratios were observed, with nearly 1:1 ratio occurring in the 23.4 and 26.1°C regimes (Table 2.2), and survivorship was approximately 4-fold higher at these temperatures (Table 2.3). Although few individuals completed development at 18.8 and 30.9°C, sex ratios were strongly male-biased at these temperatures, possibly indicating greater ability for *C. trifurcata* males to survive temperature extremes. Additionally, male-biased emergence was observed for all temperature regimes when degree day accumulations were less than 600. This may be an indication of protandry also seen in western corn rootworm, where males emerge approximately 5 d before females (Spencer et al. 2009). A male-biased sex ratio in protandrous insects may provide a greater opportunity for females to mate successfully (Del Castillo and Núñez-Farfán 2002). This behaviour was also seen in the golden-eyed lacewing (Tauber et al. 1987). However, unlike this study, sex ratio and survivorship of golden-eyed lacewing did not change with temperature, and there were no geographic differences in poikilothermic development in populations from southern Canada to Mexico (Tauber et al. 1987). Given the
differences in sex ratio and survivorship observed in this study, *C. trifurcata* in southwestern Ontario may be physiologically different from populations to the south.

The minimum developmental threshold for *C. trifurcata* was previously estimated to be 11.58 or 7.61°C for insects reared on cowpea cotyledon and soybean, respectively (Zeiss et al. 1996). These estimates are more than 1°C higher, and 2.5°C lower, respectively than the best estimate of this study. The thermal constant estimate for laboratory-reared *C. trifurcata* from egg deposit to adult emergence in this study was 628 ± 43.2°C (base 9.31°C). This is higher than the estimate of 491.0 ± 8.1 (base 11.58°C) provided by Zeiss et al. (1996). Additional estimates for first (F₁) and second (F₂) generation *C. trifurcata* degree day accumulations in field studies in Ames, IA, were 495 ± 19.6 and 542 ± 42.5, respectively (base 11.58°C), and 674 ± 28.7 and 740 ± 58.2, respectively (base 7.61°C). The difference in degree day accumulations between these studies and locations can be explained by the fact that the lower developmental threshold will dictate the amount of heat accumulated over the developmental time of the insect. The estimates from this study for thermal requirements and minimum thresholds lie within the range of estimates provided by Zeiss et al. (1996). Conversely, in Brookings, SD, degree day accumulations to complete development using a lower developmental threshold of 7.61°C (SD: F₁:540, F₂:598) (Hammack et al. 2010) were lower than those determined by this study. The reason for these differences is unclear but may be explained by the origin and environmental conditions of the *C. trifurcata* populations observed.

Differences in thermal requirements may be explained geographically, as lower developmental thresholds for insect development tend to decrease with increasing latitude (Honék 1996). Southwestern Ontario is located within similar latitudes as northern Iowa and southern South Dakota and all have humid continental climates. Despite the similarities in
latitude, one and two generations of *C. trifurcata* occur in eastern South Dakota and Iowa, respectively. If the differences between thermal constants and lower thresholds are not clearly explained geographically, the next critical step is to evaluate this model in the field.
CHAPTER THREE

Phenology of Bean Leaf Beetle, *Cerotoma trifurcata*, in Ontario and Field Validation of a Degree Day Model.

3.1 Abstract

The life cycle and voltinism of bean leaf beetle, *Cerotoma trifurcata* (Forster), was examined in three counties in 2010 and two counties in 2011 in Ontario soybean fields. Soil samples from within cages containing field-collected beetles revealed one cycle of eggs, larvae and pupae. Teneral adults were observed at only one of five locations. The three larval instars were distinguished based on head capsule width: \( L_1, 0.20-0.28; \) \( L_2, 0.30-0.40; \) \( L_3, 0.42-0.52 \) mm, respectively, and showed a clear progression from first \( (L_1) \) to third \( (L_3) \) instar. Observed degree day accumulations (DD) for *C. trifurcata* life stage events (egg hatch, egg hatch to pupation, and egg deposit to peak adult) were compared to thermal constants determined in a temperature-dependent development study. Observed and predicted DDs for all life stage events did not differ from one another. Mean degree day accumulations from first unhatched egg to peak adult emergence for the field studies was \( 589 \pm 66.8 \) DD (base 10.25°C), which was not significantly different from the model which required \( 581 \pm 39.7 \) DD (base 10.25°C). A conversion equation was used to convert predicted degree days for *C. trifurcata* for egg deposit to adult emergence to the soybean standard heat units, resulting in \( 593 \pm 40.7 \) DD (base 10°C) or \( 1068 \pm 73.2 \) DD (base 50°F).

**Key Words**  Bean leaf beetle, *Cerotoma trifurcata*, phenology, degree day model, thermal requirements
3.2 Introduction

The bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), is a known pest of soybeans, *Glycine max* (L.) Merr. (Fabaceae), and can be an economic concern for growers in Ontario. Up to three summer generations occur in the southern US (McConnell 1915, Isely 1930, Waldbauer and Kogan 1973, Loughran and Ragsdale 1986b, Smelser and Pedigo 1991, Witkowski and Echtenkamp 1996, Danielson et al. 2000, Obopile and Hammond 2001, Hammack et al. 2010), and geographically, the number of generations tends to decrease with increasing latitude (Fig. 3.1). The latitude and climate of southwestern Ontario are similar to South Dakota, Minnesota and Iowa, which have one, one with a partial second, and two generations, respectively (Loughran and Ragsdale 1986b, Smelser and Pedigo 1991, Hammack et al. 2010). However, the life cycle, voltinism and behaviour of *C. trifurcata* are unknown in Ontario and this information has important consequences for effective monitoring and control tactics in pest management programs.

Temperature and precipitation are important predictors of late-season abundance for *C. trifurcata* (Lam et al. 2001). Development of *C. trifurcata* is poikilothermic, dictated by heat accumulations which can be modelled for predictive purposes (Campbell et al. 1974, Pruess 1983). A degree day model for *C. trifurcata* (Chapter 2) developed in the laboratory using individuals originating from southwestern Ontario, differed from models published previously (Zeiss et al. 1996, Hammack et al. 2010). Differences in rates of development may be explained by shorter seasons and cooler temperatures (Honék 1996). Within a population, insects experiencing lower temperatures have delayed development (Ray 1960). Results from a study on temperature-dependent development of a species closely related to *C. trifurcata*, western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), suggested
that northern climates may increase developmental time (Schaafsma et al. 1991). However, because previous degree day models were developed in the northern range of *C. trifurcata* distribution, the differences cannot be entirely explained geographically. None of the models reported have been validated in the field for use in the new range expansion of southwestern Ontario.

Monitoring of adult *C. trifurcata* can provide insight into abundance of newly emerged and immigrating adults. However, late-season dispersal into younger host fields after senescence has begun (Pedigo and Zeiss 1996) may prevent accurate estimates of *C. trifurcata* abundance. Additionally, densities of *C. trifurcata* are influenced by survival and developmental rates of immature stages, which may vary according to location, soil type and moisture content (Marrone and Stinner 1984). For example, larval development was reduced and survivorship was increased in wet and organic soils in the eastern Coastal Plain of North Carolina, where population levels were the largest in the state (Marrone and Stinner 1984). Understanding the reproductive success of *C. trifurcata* in southwestern Ontario requires that immature stages, which are subterranean, and adults should both be sampled. Efficient extraction techniques for subterranean immatures have been developed to sample eggs, larvae and pupae (Waldbauer and Kogan 1973, 1975, Anderson and Waldbauer 1977, Levinson et al. 1979).

The objectives of this study were to 1) determine voltinism of *C. trifurcata* in southwestern Ontario, and 2) validate the laboratory degree day model for *C. trifurcata* life stage events in the field.
Figure 3.1. Number of *C. trifurcata* generations in the United States and field study site locations in southwestern Ontario in 2010 and 2011. Ontario study site locations: 1) Bruce county 2010/Huron county 2011, 2) Wellington county 2010, 3) Municipality of Chatham-Kent.

3.3 Materials and Methods

3.3.1 Field Cage Studies

*C. trifurcata* adults were confined in cages in commercial soybean fields and research plots to determine the occurrence of life stage events throughout the growing season and the number of generations that occur in southwestern Ontario. In 2010, one northern, intermediate and southern location was chosen in southwestern Ontario; represented by Bruce County, Wellington County and the Municipality of Chatham-Kent, respectively (Fig. 3.1). The 2011 study was conducted in northern Huron County and the Municipality of Chatham-Kent only, as beetles were not found in Wellington County early in the season. The 2010 Bruce and 2011 Huron county study sites were ~ 18 km apart, and the study sites in Chatham-Kent for 2010 and 2011 were ~ 24 km apart. Soybean fields planted with non-insecticide treated seed were selected as study sites.

There were two sizes of cages available for the study: the smaller cage was 60 x 91.25 cm base and 86.25 cm tall, and the larger cage was 91 x 91 cm base and 91 cm tall (fabricated using pressure-treated wood and No-see-um mosquito netting, Seattle Fabrics Inc., Seattle, Washington, US). Cages were placed in a soybean field at each location within a few weeks of soybean emergence. In 2010, six of the smaller cages were used at each location, while in 2011, 11 smaller cages in Huron and 10 smaller cages in Chatham-Kent were used. All locations had two of the larger cages. Plant stand within cages was thinned to 15 plants and cages were infested with 15 field-collected beetles (one beetle/plant). The ratio of males:females used for infestations was based on natural populations sampled in the corresponding counties. Detail for location, infestation date and number of male and female *C. trifurcata* adults used in infestations are presented in Table 3.1.
Table 3.1. Summary of infestation dates, sampling completion dates and number of male and female *C. trifurcata* used for field cage trials at 5 locations over 2 years.

<table>
<thead>
<tr>
<th>Year</th>
<th>Site</th>
<th>GPS Co-ord.</th>
<th>Male:Female</th>
<th>Infestation date</th>
<th>Last sample date</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>Bruce</td>
<td>44.032</td>
<td>2:13</td>
<td>18-Jun</td>
<td>15-Sep</td>
</tr>
<tr>
<td>2010</td>
<td>Wellington</td>
<td>43.706</td>
<td>2:13</td>
<td>21-Jun</td>
<td>17-Sep</td>
</tr>
<tr>
<td>2010</td>
<td>Chatham-Kent</td>
<td>42.448</td>
<td>6:9</td>
<td>01-Jul</td>
<td>21-Sep</td>
</tr>
<tr>
<td>2011</td>
<td>Huron</td>
<td>43.874</td>
<td>4:11</td>
<td>07-Jul</td>
<td>15-Sep</td>
</tr>
<tr>
<td>2011</td>
<td>Chatham-Kent</td>
<td>42.447</td>
<td>6:9</td>
<td>30-Jun</td>
<td>15-Sep</td>
</tr>
</tbody>
</table>
3.3.2 Soil Sampling

At each sampling interval, one small cage was sampled destructively by removing the cage, cutting the soybean plants at the soil surface and collecting surrounding soil and soybean roots. Sampling occurred every 10-14 d in 2010. In 2011, sampling intervals were shortened to 3-4 d to increase accuracy of pupal and larval life stage estimates. In 2010, soybean roots for all 15 plants and surrounding soil were collected from the entire area of the cage to a depth of ~ 10 cm. In 2011, to reduce processing time and because 92% of larvae are found in the top 7.6 cm of soil (Anderson and Waldbauer 1977, Kogan and Herzog 1980), there were two sampling intervals per cage consisting of 7-8 adjacent plants and surrounding soil was collected to a depth of ~ 7 cm and a horizontal distance of ~ 5 cm from the row. Soil sample volumes ranged from approximately 4-82 L in 2010 and 1.8-6.2 L in 2011. Samples were stored at ~ 4°C until processing.

3.3.3 Egg, Larval and Pupal Recovery

Soil samples were processed using the Illinois Egg Separator technique (Waldbauer and Kogan 1973) with some modifications. Larvae and pupae found floating on the surface of a brine solution after samples were soaked for 1-2 h were removed before samples were washed through a 20- and 100-mesh sieve tower (Fisherbrand®, Thermo Fisher Scientific Inc., Waltham, MA). The soil debris remaining in the 20-mesh sieve was inspected for 3rd instar larvae and pupae before discarding. The remaining soil in the 100-mesh sieve was placed in a separatory funnel with brine solution to extract eggs, and 1st and 2nd instars by flotation. An aquatic pump was used to agitate the mixture to keep eggs and small larvae from attaching to debris. A baffle was not necessary because a vortex did not form with the equipment used.

A dissecting scope was used to examine the material floating in the separator funnel.
Eggs (unhatched in 2010; hatched and unhatched in 2011), larval and pupal counts were recorded with samples preserved in 70% ethanol. The number of eggs, larvae and pupae (standardized by soil volume) extracted, and teneral adults emerging in cages were recorded at the specified sampling intervals expressed as days after infestation (DAI) and calendar day (CD). Larval head capsule widths (Isely 1930) were measured from larvae recovered from soil samples at 4x magnification, using a dissecting microscope fitted with a micrometer eyepiece.

3.3.4 Field Validation of the Degree Day Model

Degree day models developed through constant temperature growth chamber studies (Chapter 2) were validated using temperature and life stage data from this field study. Air temperature and relative humidity (0.7 m above ground in 2010 and 1 m above ground in 2011) (Hobo® Pro Series Model H08-032-08 used in 2010; Model U23-001 Pro V2 used in 2011) and soil temperature and moisture (7 cm below ground) (Hobo® micro station Model H21-002 with 12-bit soil temperature sensor) were recorded at each location (1 cage in 2010 and 2 cages in 2011). Temperatures were recorded every 30 min. from time of artificial infestation until the study was complete. Temperature records could not be retrieved in 2010 from the air and soil temperature logger at the site in Chatham-Kent, and the soil temperature logger at the site in Bruce County due to malfunction. Weather records from the closest proximal weather station were used for the site in Chatham-Kent and air temperature recorded within cages was used for the site in Bruce County in 2010. A descriptive summary of temperature records is presented in Table 3.2. Degree day accumulations were calculated using the best estimate of the lower developmental threshold, 10.25°C (Chapter 2). Records of first unhatched egg to first larva, first larva to first pupa, and first adult to adult emergence were considered life stage events for egg hatch, egg hatch to pupation and egg hatch to adult emergence. Pupal duration could not be
Table 3.2. Summary of temperature records used in analyses of *C. trifurcata* development after field cage trials at 5 locations over 2 years.

<table>
<thead>
<tr>
<th>Year</th>
<th>Site</th>
<th>Temperature Type</th>
<th>Mean T</th>
<th>SD</th>
<th>T&lt;sub&gt;Max&lt;/sub&gt;</th>
<th>T&lt;sub&gt;Min&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>Chatham-Kent</td>
<td>air&lt;sup&gt;1&lt;/sup&gt;</td>
<td>21.5</td>
<td>3.22</td>
<td>30.0</td>
<td>10.0</td>
</tr>
<tr>
<td>2010</td>
<td>Wellington</td>
<td>soil</td>
<td>19.0</td>
<td>3.45</td>
<td>31.2</td>
<td>11.1</td>
</tr>
<tr>
<td>2010</td>
<td>Bruce</td>
<td>air&lt;sup&gt;2&lt;/sup&gt;</td>
<td>20.0</td>
<td>5.81</td>
<td>38.3</td>
<td>4.2</td>
</tr>
<tr>
<td>2011</td>
<td>Chatham-Kent</td>
<td>soil</td>
<td>21.6</td>
<td>3.63</td>
<td>36.1</td>
<td>10.5</td>
</tr>
<tr>
<td>2011</td>
<td>Huron</td>
<td>soil</td>
<td>19.4</td>
<td>3.12</td>
<td>31.4</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Due to malfunction of data loggers, air temperatures were used from ¹the closest proximal weather station and ²records within cages.
estimated because teneral adults were only observed at one location and on the same date as a first pupal record.

To validate the degree day model from egg deposit to adult emergence, trap captures from three other soybean fields (Brunke 2011) and adult emergence from the Wellington County site for the current study were used. Oviposition was assumed to begin shortly after adults colonized a soybean field, so degree day accumulations commenced on the date when beetles were first recorded at a given location. Total degree day accumulations from egg deposit to adult emergence in the field were considered to be from time of first adult to peak adults. The closest proximal weather station data were used to calculate daily degree day accumulations at each location.

To satisfy assumptions of normality and achieve linearity in regression analyses, data were transformed based on the highest Shapiro-Wilk statistic. Degree day accumulations were subjected to ANOVA using the PROC MIXED procedure (SAS 9.2, SAS Institute, Cary, NC). Data were analyzed separately for each year because sampling interval and depth differed between years. Thermal constant estimates (i.e. predicted degree day accumulations) (Chapter 2) were converted to a standard base 10.25°C using a linear regression equation (PROC GLM) as a conversion factor according to methods determined by Pruess (1983). Predicted degree day accumulations were also converted to base 10°C and 50°F, in order to express degree day accumulations on the same scale as soybean standard heat units (Brown 1960). Mean degree day accumulations (base 10.25°C) were compared with predicted degree day accumulations for all life stage events using Welch’s t-test (Sokal and Rohlf 1969).
3.4 Results

3.4.1 Phenology

Eggs and larvae were recovered from all 5 sites over the 2 years of the study and pupae were recovered from all sites except from Chatham-Kent in 2010 (Figs. 3.2 & 3.3). Teneral adults were observed only at the Wellington site in 2010 (Fig. 3.2). In 2010, peak egg deposit in Bruce County occurred 16 and 9 d later than in Wellington County and Chatham-Kent, respectively, with a second peak occurring towards the end of the sampling period at all three locations (Fig. 3.2). In 2011, two strong peaks for egg laying were observed at both locations; the first occurred 3-5 wk earlier than in 2010 (Fig. 3.3). The first peak of eggs in 2010 occurred less than 3 DAI earlier than the second peak in the southern and northern sites in 2011. Small numbers of larvae were recovered towards the end of the sampling period in 2010, although only one peak, spanning 13-22 d, was observed at all 3 locations. In 2011, the two larval peaks occurred 5 and 11 d later in Huron than in Chatham-Kent. A third larval peak occurred in Chatham-Kent in 2011. In 2010, few pupae were recovered from the Bruce County site, but a clear peak was observed at the Wellington site corresponding to a declining numbers of larvae at that site. Peak pupal recovery in 2011 at Huron County and the Municipality of Chatham-Kent occurred 38 and 20 d after the first larval recovery peak, respectively. While egg and larval peaks tended to occur 5-12 d earlier in the southern locations, the pupal peaks for southern locations in both years occurred 7-9 d later than in the northern counties. The greater number of peaks of eggs and larvae observed in 2011 than in 2010 may be attributed to shorter sampling intervals. Teneral adults from two cages at the Wellington site in 2010 began emergence the same date that pupae were first recovered, but adult numbers peaked 11 d later (Fig. 3.2). Photographs of hatched and unhatched eggs, a larva and a pupa are presented in Fig. 3.4.
Figure 3.2. Abundance of *C. trifurcata* eggs, larvae and pupae per cm³ soil and teneral adults per cage at three locations in southwestern Ontario in 2010 from field cage trials in soybean fields.
Figure 3.3. Abundance of *C. trifurcata* eggs, larvae and pupae per cm³ soil at two locations in southwestern Ontario in 2011 from field cage trials in soybean fields.
Figure 3.4. Immature *C. trifurcata* recovered from soil samples after adults were confined in field cage studies in three counties in southwestern Ontario in 2010. Sample photographs are (A) hatched eggs and (B) unhatched eggs from the Bruce County site on 13 July, 2010; (C) larva from the Wellington site on 2 August, 2010, and (D) pupa from the Wellington site on 16 August, 2010. Photographs were taken by Heather Cumming and Dave Cheung in the University of Guelph Insect Systematics Lab.
3.1.1 Larval Head Capsule Measurements

Head capsule widths ranged from 0.20-0.55 mm. Specimens were classified according to head capsule width to allow the three larval instars to be distinguished: L₁, 0.20-0.28; L₂, 0.30-0.40; L₃, 0.42-0.52 mm, respectively. Several specimens were damaged during the processing of soil samples and head capsules could not be measured. Weekly abundance of each instar was determined at each site throughout the sampling period (Fig. 3.5). No first instar larvae were recovered from Chatham-Kent in 2010. All sites showed a clear progression over time from first to third instars. Most recovery of first, second and third instars occurred before week 6, between week 3 and 7, and after week 5, respectively. The number of first and second instars peaked simultaneously in 3 of 5 sites. The number of third instars peaked after those for first and second instars at all 5 sites. Regardless of location, year or infestation date, few first or second instar larvae were found after 214-220 CD (i.e. 3-8 August). Three of five sites showed a linear trend of head capsule size increasing with DAI ($P < 0.05$) (Fig. 3.6).

3.1.2 Degree Day Model Field Validation

The duration of life stage did not differ between years for first unhatched egg to first larva ($F=8.71$, $df=1,3$, $P=0.060$) and first larva to first pupa ($F=0.06$, $df=1,2$, $P=0.831$) (Table 3.3). Degree day accumulations between years were not significantly different for first unhatched egg to first larva ($F=5.99$, $df=1,3$, $P=0.092$) and first larva to first pupa ($F=0.24$, $df=1,2$, $P=0.6705$) (Table 3.3). Regressions used to convert predicted degree days for life stage events (Chapter 2) to standard degree days using the lower developmental threshold 10.25°C are presented in Fig. 3.7. Egg hatch for 2010 and 2011 occurred 9 and 71 d earlier, respectively, and egg hatch to pupation in 2010 occurred 36 d earlier than predicted by the model, but observed and predicted values did not significantly differ (Table 3.4). The largest discrepancy between
Figure 3.5. Abundance of larval instars per cm³ based on head capsule size after field cage trials at five locations in 2010 and 2011. Head capsule ranges for first, second and third instars were: 0.20-0.28, 0.30-0.40 and 0.42-0.52 mm, respectively.
Figure 3.6. Linear regression and observed larval head capsule width (mm) from specimens recovered from soil samples after *C. trifurcata* field cage trials at three locations in Ontario in 2010 and 2011.
Table 3.3. Mean number of developmental days (± SE) and mean degree day accumulations (± SE) (base 10.25°C) between *C. trifurcata* life stage events in field cage trials at Bruce, Huron and Wellington counties and the Municipality of Chatham-Kent in 2010 and 2011.

<table>
<thead>
<tr>
<th>Year</th>
<th>First egg to first larva (Mean ± SE)</th>
<th>First larva to first pupa (Mean ± SE)</th>
<th>First egg to first adult¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
<td>DD (base 10.25°C)</td>
<td>Days</td>
</tr>
<tr>
<td>2010</td>
<td>11.0 ± 1.18a</td>
<td>139.1 ± 16.14a</td>
<td>28.5 ± 7.29a</td>
</tr>
<tr>
<td>2011</td>
<td>5.5 ± 1.44a</td>
<td>76.7 ± 19.76a</td>
<td>26.0 ± 7.29a</td>
</tr>
</tbody>
</table>

¹Results for first egg to first adult are not means because adult emergence was only observed at the Wellington site. Means within columns followed by the same letter are not significantly different (P ≥ 0.05).
Figure 3.7. Linear regressions of degree day accumulations using different lower developmental thresholds (base temperatures) calculated from temperature data at five sites from 7 July to 15 September in southwestern Ontario in 2010-2011. Lower developmental thresholds used were based on those determined from linear regressions for development of *C. trifurcata* life stage events (Chapter 2) and all were regressed against the best estimate determined in the temperature-dependent development study (10.25°C) (Chapter 2). (A) Base 9.25°C for pupation; (B) Base 9.47°C for adult emergence; (C) Base 9.31°C for egg deposit to adult emergence.
Table 3.4. Comparison of observed mean degree day requirements (base 10.25°C) versus predicted estimates for *C. trifurcata* life stage events for egg hatch and egg hatch to pupation and egg deposit to peak adults in Ontario soybean fields based on sampling from pan and canopy traps in 2009 in Auburn and Benmiller (Brunke 2011), and field cage trials in 2010 and 2011 in Ridgetown.

<table>
<thead>
<tr>
<th>Life stage</th>
<th>Year</th>
<th>DD (base 10.25°C)</th>
<th>Difference (O - P) DD</th>
<th>t-value (df)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Predicted&lt;sup&gt;¹&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg hatch&lt;sup&gt;²&lt;/sup&gt;</td>
<td>2010</td>
<td>139</td>
<td>148</td>
<td>-9</td>
<td>0.5237 (2)</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>77</td>
<td>148</td>
<td>-71</td>
<td>3.5429 (1)</td>
</tr>
<tr>
<td>Egg hatch to pupation&lt;sup&gt;²&lt;/sup&gt;</td>
<td>2010</td>
<td>284</td>
<td>320</td>
<td>-36</td>
<td>0.2999 (2)</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>367</td>
<td>320</td>
<td>46</td>
<td>0.3879 (1)</td>
</tr>
<tr>
<td>Egg deposit to peak adult</td>
<td>2009&lt;sup&gt;³&lt;/sup&gt;-2010&lt;sup&gt;⁴&lt;/sup&gt;</td>
<td>589</td>
<td>581</td>
<td>8</td>
<td>0.1482 (9)</td>
</tr>
</tbody>
</table>

<sup>¹</sup>Predicted values were converted to degree days (base 10.25°C) based on the best estimate from the temperature-dependent development study (Chapter 2).
<sup>²</sup>Field cage trials. <sup>³</sup>Sampling from pan and canopy traps using observations of first adults under the assumption that adults immediately oviposit after colonizing a soybean field. <sup>⁴</sup>*In situ* counts after field cage trials.
observed and predicted values was 71 DD (base 10.25°C) from 2011 egg hatch, which equates to about 5 d based on average daily degree day accumulations observed during this stage in 2011 (Table 3.3). The predicted model for *C. trifurcata* to complete development from egg to adult (581 ± 39.7 DD) (base 10.25°C) did not significantly differ from observed DD accumulations from first adult to peak adult in the field (589 ± 66.8) (base 10.25°C) (Table 3.4). Using the conversion equation in Fig. 3.8, predicted degree days for *C. trifurcata* from egg deposit to adult emergence are 593 ± 40.7 DD (base 10°C) or 1068 ± 73.2 DD (base 50°F) (Fig. 3.8).

### 3.2 Discussion

Only one generation of *C. trifurcata* was observed in southwestern Ontario, with some evidence of early stages of a second generation similar to that observed in eastern South Dakota (Hammack et al. 2010). Degree day accumulations from egg deposition to adult emergence support the conclusion of only one generation, and the progression of larval instars throughout the growing season also indicated one complete cycle of larval development. Natural populations of *C. trifurcata* were observed in Bruce (2010) and Huron (2011) sites before cages were installed, which may explain why larger specimens were recovered in early samples (Fig. 3.6). Two peaks in egg and larval recovery were common in this study, however, only one clear pupal peak was observed. Regardless of infestation date, for all sites, the majority of pupae were recovered between 4 and 16 August and few were recovered after 29 August, while the second peak in egg and larval recovery occurred from late August to early September. Given that teneral adults began emerging 16 August, it is likely that the second peak in eggs and larvae were a result of the first summer generation mating and oviposition. However, degree day accumulations after the second peak were insufficient to support complete development of a past,
Figure 3.8. Linear regressions of degree day accumulations using the standard base temperature used for soybean heat units (10°C) against the lower threshold for egg deposit to adult emergence (9.31°C) determined from the temperature-dependent development study (Chapter 2).
second generation. Although, *C. trifurcata* were unable to complete a second generation in the current study, during a warmer season, with optimum growing conditions, that potential exists. Some climate change experts have predicted that European corn borer, *Ostrinia nubilalis* (Hübner), will establish up to 1220 km north of their current range (Porter et al. 1991). In the past, other species such as the southwestern corn borer, *Diatraea grandiosella* Dyar, a native of subtropics, have adapted to cooler climates and harsher winters in the US (Chippendale et al. 1992). On average over two years of study, *C. trifurcata* would require ~ 350 DD (base 10.25°C) more to complete a second generation in southern Ontario. However, heat accumulated rapidly in Chatham-Kent during the growing season of 2011 and this number was reduced to 170 DD (base 10.25°C). Monitoring and management programs in southwestern Ontario should consider the possibility of additional range expansion and multiple summer generations of *C. trifurcata* under future climates.

*C. trifurcata* development in the field supported observations made in growth chambers. The first and peak adult emergence from within cages at the Wellington County site in 2010 was observed after 56 and 67 d, respectively. Under the assumption that adults began laying eggs immediately after being placed in the cage, the number of days to complete development from egg to adult in the field, lies within the range of days observed at 18.8 and 23.4°C, occurring at 68 and 45 d, respectively (Chapter 2). Moreover, average temperatures in the field cage studies fell within this temperature range of 19.0 to 21.6°C, suggesting that this range was a suitable comparison of temperatures experienced by immature *C. trifurcata* in southern Ontario.

In southwestern Ontario, adult populations peaked from mid-August to early September. In Minnesota, where there is also one generation, teneral adults were observed as early as mid-July, but peaked during late August (Loughran and Ragsdale 1986b). In Iowa, however, the second
generation adults were abundant in mid to late August (Smelser and Pedigo 1991). Although these locations are at similar latitudes, differences in voltinism may be explained by the length of the growing season. Overwintered beetles were found colonizing soybeans in early May in Iowa (Smelser and Pedigo 1991), but planting in Minnesota and southwestern Ontario typically occurs several weeks later (Loughran and Ragsdale 1986b). The additional availability of host plants at the beginning and end of the growing season may be enough to sustain a second summer generation in Iowa.

Head capsule measurements in this study for first, second and third instars were 1.1-6.2% wider than those previously determined by Isely (1930). Climatic differences between South Carolina (the location of Isely’s study) and southwestern Ontario may explain the larger head capsule widths observed in this northern region. Climatic differences can explain geographical differences in size in several cold-blooded species (Ray 1960). For example, head capsule width and wing length of the mosquito, *Anopheles merus* Dönitz, decreases with increasing temperature (Sueur and Sharp 1991).

The occurrence of a single generation of *C. trifurcata* in southwestern Ontario has important implications for pest management programs, and the soybean-colonizing population should become the primary target of control efforts. Although high populations of adults and larvae feeding through the vegetative stages can have harmful effects on soybean yield, late-season pod-feeding during the reproductive stages damages the harvestable part of soybean crop and may cause significant reductions in yield and quality. Therefore, the most critical events to control are field colonization by overwintered adults and late-season pod-feeding. The timing of soybean colonization is crucial to the reproductive success of the overwintered population. When colonization is not synchronized with soybean planting, low populations and little damage
may be observed (Jeffords et al. 1983). The use of seed treated with insecticides can reduce the initial colonizing population (Witkowski and Echtenkamp 1996) and provide adequate control for insects with a single summer generation (Koch et al. 2005). In Ontario, use of insecticidal seed treatments has increased and appears to correspond with a decline in *C. trifurcata* populations, though economically damaging populations are still known to occur.

Given that there is only one summer generation of *C. trifurcata* in southwestern Ontario, the degree day model provided here may be useful to predict adult emergence which often coincides with a critical point in soybean development: pod formation. Crop heat units (CHU) (i.e. degree days) are used by soybean breeders and growers to determine the proper variety to be grown in a particular region. Therefore, when degree day accumulations approach 500 (base 10°C); growers should implement scouting to monitor *C. trifurcata* populations and prepare for the possibility of foliar insecticide treatments. To optimize the timing of control actions, pod-feeding action thresholds are needed.
CHAPTER FOUR

Impact of Late-Season Pod-Feeding by Cerotoma trifurcata on Yield and Quality of Soybean in Ontario.

4.1 Abstract

Field cage studies were conducted to evaluate the effect of late-season pod-feeding of bean leaf beetle, Cerotoma trifurcata, on yield and quality of soybean in Ridgetown, Ontario in 2010-11. Defoliation increased with beetle number, reaching a maximum of 22.5% at 16 beetles per plant. Percentage of pods with feeding lesions, number of lesions per pod and number of pod lesions per plant increased with number of beetles per plant and soybean reproductive stage for both years. There was also a positive correlation between number of beetles per plant and soybean reproductive stage, with number of stem lesions per plant in 2010 and number of clipped pods per plant in 2011. Seed quality assessments revealed a significant increase in percentage of damaged, insect damaged, immature, mouldy dark, stained brown, stained black and shrivelled seed with number of beetles per plant in 2011. Only three of 176 samples over two years of study tested positive for bean pod mottle virus (BPMV) by enzyme-linked immunosorbant assay (ELISA). Yield from cages containing 16 beetles per plant were significantly lower than from caged and uncaged controls. Yield decreased by 1.24% for every additional beetle per plant in 2011 and by 59.8 kg/ha for every additional beetle per plant, when data for both years were combined. Economic injury levels ranged from 0.34 to 2.50 beetles per plant or 10 to 75 beetles per m of row.
Key words: Bean leaf beetle, *Cerotoma trifurcata*, pod-feeding, soybean reproductive stage, economic injury level

4.2 Introduction

The bean leaf beetle, *Cerotoma trifurcata* (Forster), is an economic pest of soybean, *Glycine max* (L.) Merr. (Fabaceae), in the US (Chittenden 1897, Kogan and Herzog 1980, Smelser and Pedigo 1992c). Adult feeding causes defoliation and lesions on stems, pods and peduncles of soybean, resulting in up to 50% yield losses (Kogan and Herzog 1980, Smelser and Pedigo 1992c, b).

*C. trifurcata* is the primary vector of the bean pod mottle virus (BPMV), *Comovirus* (Comoviridae), for which symptoms include foliar mosaic, mottling of seeds and pods, stunting of plants and necrosis (Ross 1968, Giesler et al. 2002, Hobbs et al. 2003, Zheng et al. 2005a). Direct yield losses from BPMV infection can reach 50% (Hopkins and Mueller 1984) and the presence of this virus was confirmed in Ontario soybean fields in 2011 (Michelutti et al. 2002). Another virus that presents itself with similar symptoms is the soybean mosaic virus (SMV), *Potyvirus* (Potyviridae), which is vectored by the soybean aphid, *Aphis glycines* Matsumura (Ross 1968, 1969). When BPMV and SMV are present simultaneously, there can be an additive effect on yield with losses reaching 80% (Ross 1968, Tu et al. 1970, Hobbs et al. 2003). In addition, yield and quality may be reduced by secondary infections of fungal pathogens (Shortt et al. 1982, Obopile and Hammond 2001).

Seed quality is especially important for soybean grown for the identity preserved (IP) food grade market, where downgrading occurs for damaged and/or discoloured seed. The Canadian Grain Commission (2010) establishes tolerances for each of these grading factors.
within its published grade standards (i.e. 0.2% heated or mouldy seed, or 3% damaged seed). Buyer specifications for IP and food grade soybeans demand the highest quality and there is little to no tolerance for damaged grain (D. Buttenham⁵, personal communications).

Economic thresholds for *C. trifurcata* late-season pod-feeding were evaluated by Smelser & Pedigo (1992c, b), however, these studies were conducted in Ames, IA, US, where the beetle has 2 generations per year. In Ontario, *C. trifurcata* only undergoes one complete summer generation (Chapter 3) and the timing and extent of feeding may differ from that observed in the US. Furthermore, the thresholds developed by Smelser and Pedigo (1992c, b) are applicable during the R6 reproductive stage (Fehr et al. 1971). To my knowledge, no studies have compared the impact of feeding during different stages of pod development, nor has *C. trifurcata* pod-feeding of soybean been evaluated in southwestern Ontario.

The objectives of this study were to 1) evaluate the impact of adult *C. trifurcata* feeding during pod formation on yield and quality, and 2) determine economic injury levels for southwestern Ontario.

### 4.3 Materials and Methods

#### 4.3.1 Field Cage Studies

Populations of *C. trifurcata* adults at several densities were confined in cages containing soybean plants during pod formation reproductive stages (R-stage) (Fehr et al. 1971) to produce insect density and plant stage-dependent data for soybean yield and quality. This study used a split-plot design replicated four times, with R-stage (R3, R4, R5 and R6) as the main effect and number of beetles per plant as the subplot effect. In 2010, beetle treatments were 0 (caged

---

⁵ D. Buttenham, Secretary-Manager, Canadian Soybean Exporters’ Association, Guelph, ON, Canada
control), 2, 4 and 8 beetles per plant and an uncaged control. In 2011, beetle treatments were changed to 0 (caged control), 4, 8, and 16 beetles per plant and an uncaged control. Reproductive stages used in this study are defined as follows: R3-4 included pod development and R5-6 included seed development (Fehr et al. 1971). Cages (75 x 75 cm base, 115 cm tall, BugDorm, Model 2400, MegaView Science Co. Ltd., Taichung, Taiwan) were placed in an identity preserved (IP) soybean (cv. Pinehurst) field with 76 cm rows in Ridgetown, ON, Canada, when > 50% of plants in the field had reached the corresponding R-stage. When > 80% of the entire field had progressed into the next R-stage, treatments were considered complete for that R-stage and cages were removed from the plots. In 2010, plant populations were thinned to 5 per cage prior to cage placement (i.e. the beginning of each R-stage). Soybean plots that were thinned at earlier developmental stages may have been able to increase pod production (Probst 1945) compared to those thinned at later stages. Therefore in 2011, all plots were thinned at the same time during the late vegetative stages. Entire fields were monitored for natural populations of C. trifurcata and A. glycines throughout the season. Both caged and uncaged control plots were sprayed weekly with cyhalothrin-lambda (Matador, 9.96 g a.i./ha, Syngenta Crop Protection Inc., Guelph, ON) after the first sign of any insect activity. During both years, low populations of A. glycines were observed in the field. To reduce impact from secondary pests, all plots were sprayed after cages were removed.

4.3.2 Feeding Damage Assessments

After cages were removed, plants were assessed for percentage of defoliation in the upper, middle and lower canopy, number of injured pods, number of pod and stem lesions and extent number of clipped pods. Pods were counted as clipped when there was clear feeding damage and the peduncle was compromised. A defoliation index was used to assess defoliation
visually (Ontario Ministry of Agriculture Food and Rural Affairs 2011b).

4.3.3 Yield and Quality Assessments

Yield was assessed by hand harvesting all plants in each plot at maturity and obtaining seed using a single plant thresher. Plot weights adjusted to 14% moisture were used to evaluate seed yields. Seed samples were counted and quality was assessed according to the Canadian Grain Commission Official Grain Grading Guide (2010). All seeds from each plot were sorted into the following categories: total damaged (DMG), insect damaged (IDMG), immature (IM), mouldy white (MLDYW), mouldy dark (MLDYD), stained brown (STNDBR), stained black (STNDBL), mottled purple (MOTTPUR), mottled brown (MOTTBR), and shriveled (SHRVL). Percentage of seed in each category was determined.

After quality analysis was completed, seed samples were finely ground and 5 g samples were brought to the Pest Diagnostic Clinic at the Agriculture and Food Laboratory, University of Guelph, Guelph, ON, where an enzyme linked immunosorbent assay (ELISA) was performed on a 0.5 g sample to determine presence of bean pod mottle virus (BPMV). BPMV antibody and its enzyme conjugate were supplied by Agdia (Elkhart, IN).

4.3.4 Economic Threshold

Yield per plot for each year and both years combined was regressed against number of beetles per plant to develop a model for yield reduction caused by C. trifurcata based on methods of Hammond and Pedigo (1982). Gain thresholds were calculated for non-IP and IP soybeans (equations 1 and 2, respectively),

\[
gain \text{ threshold (kg/ha)} = \frac{\text{management costs (\$/ha)}}{\text{soybean market value (\$/ha)}} \quad [1]
\]

\[
gain \text{ threshold (kg/ha)} = \frac{\text{management costs (\$/ha)}}{\text{soybean market value + IP premium (\$/ha)}} \quad [2]
\]
and used to calculate economic injury levels (EILs),

\[
EIL \text{ (insects/ha)} = \frac{\text{gain threshold (kg/ha)}}{\text{loss per insect (kg/insect)}}
\]

Market values and insecticide costs were obtained from the Grain Farmers of Ontario (N. Mackellar\(^6\), personal communications) and local elevators and suppliers (Syngenta Crop Protection Inc., http://www.syngenta.com, and South West Ag Partners Inc., http://www.southwestag.ca). Premiums for IP soybeans were obtained from the Canadian Soybean Exporters’ Association (D. Buttenham\(^7\), personal communications). Premiums for a standard IP soybean are $0.07/kg ($2.00/bu). For large-seeded (high protein) and small-seeded soybeans, higher premiums of $0.17-0.18/kg ($4.50-5.00/bu) are offered, although these varieties tend to have lower yields (D. Buttenham\(^5\), personal communications). A range of market values was used that encompassed non-IP and IP soybeans prices over the last 5 years. Average yields in Ontario were obtained from the Ontario Ministry of Agriculture Food and Rural Affairs (Ontario Ministry of Agriculture Food and Rural Affairs 2013). Based on current retail insecticide prices, a range of pest management costs was used that reflects various combinations of management practices. EILs were calculated for number of beetles per plant and number of beetles per metre of row.

4.3.5 Statistical Analysis

To satisfy assumptions of normality and achieve linearity in regression analyses, data were transformed based on the highest Shapiro-Wilk statistic, as follows: natural log transformations were used for 2010 data on percentage of immature, mouldy dark, stained brown and shrivelled seed and number of stem lesions per plant, and 2011 data on percentage of immature, mouldy dark, stained brown, mottled brown and shriveled seed, percentage of pods

\(^6\) N. Mackellar, Market Development Coordinator, Grain Farmers of Ontario, Guelph, ON, Canada
\(^7\) D. Buttenham, Secretary-Manager, Canadian Soybean Exporters’ Association, Guelph, ON, Canada
with feeding damage, number of pod lesions per plant, and number of stem lesions per plant; and arcsine square root transformations were used for 2010 data on percentage of damaged, insect damaged, mouldy white, stained black, mottled brown, and mottled purple seed, percentage of pods with feeding damage, number of lesions per pod, percentage of defoliation, number of lesions per plant and number of clipped pods per plant; and square root transformations were used for 2011 data on percentage of damaged and stained brown seed and number of lesions per pod. Data were subjected to ANOVA using PROC MIXED (SAS 9.2, SAS Institute, Cary, NC). Fixed effects were R-stage, number of beetles and an interaction between R-stage and number of beetles. Random effects were replicate and replicate by stage interaction. If there was a significant effect of R-stage, a subsequent analysis was performed with R-stage as the fixed effect which excluded the caged and uncaged controls. If there was no interaction between R-stage and number of beetles per plant, then a subsequent regression analysis was performed on number of beetles per plant and number of feeding days against the independent variables using PROC GLM (SAS 9.2, SAS Institute, Cary, NC). The number of potential feeding days was calculated as number of beetles per plant x number of days in R-stage. When a significant cage effect was determined by ANOVA, the uncaged control was not included in the regression analysis. A percentage of yield reduction index was calculated by subtracting beetle treatment yields from the caged control for each replicate. Predicted values from transformed regression coefficients and means, were back-transformed to their original scale. Means separations were conducted using Fisher’s Protected Least Significant Difference (LSD) ($P < 0.05$).
4.4 Results

4.4.1 Feeding Damage Assessments

In 2010 and 2011, there were no significant interactions between number of beetles per plant and R-stage for percentage of defoliation. However, there were significant effects on percentage of defoliation from number of beetles and R-stage for both years (Figs. 4.1 and 4.2). In 2010, defoliation was lowest during R3 and highest at R4 ($F = 9.07$, df = 3,57, $P < 0.0001$) (Fig. 4.1). Percentage of defoliation decreased significantly with R-stage in 2011 ($F = 17.73$, df = 3,57, $P < 0.0001$). When the caged and uncaged controls were removed from the analysis, a similar trend was observed for both years (2010: $F = 4.34$, df = 3,41, $P = 0.0096$; 2011: $F = 3.58$, df = 3,41, $P = 0.0218$). In both years, defoliation significantly increased with number of beetles per plant (2010: $F = 82.31$, df = 4,57, $P < 0.0001$; 2011: $F = 219.42$, df = 4,57, $P < 0.0001$) (Fig. 4.2), reaching a maximum of 22.5% at 16 beetles per plant. A significant cage effect was observed in 2011, with significantly more defoliation occurring in the uncaged than the caged control. In both years there was a significant relationship between percentage of defoliation and number of beetles per plant (2010: $R^2 = 0.693$, $P < 0.0001$; 2011: $R^2 = 0.696$, $P < 0.0001$) and with number of feeding days (2010: $R^2 = 0.588$, $P < 0.0001$; 2011: $R^2 = 0.238$, $P < 0.0001$).

In both years, there was an interaction between number of beetles per plant and R-stage for percentage of pods with feeding damage (2010: $F = 2.79$, df = 12,57, $P = 0.0047$; 2011: $F = 9.34$, df = 12,57, $P < 0.0001$), number of lesions per pod (2010: $F = 5.9$, df = 12,57, $P < 0.0001$; 2011: $F = 10.77$, df = 12,57, $P < 0.0001$) and number of pod lesions per plant (2010: $F = 5.69$, df = 12,57, $P < 0.0001$; 2011: $F = 10.92$, df = 12,57, $P < 0.0001$) (Fig. 4.3 and 4.4). Significant interactions between number of beetles per plant and R-stage were found for number of stem lesions per plant in 2010 ($F$-value = 1.93, df = 12,57, $P = 0.049$) (Fig. 4.3) and number of
Figure 4.1. Mean percentage of defoliation (± 95% CI) from field cage studies of adult *C. trifurcata* during pod development of soybeans in Ridgetown, ON, 2010-11. Treatments with different numbers of beetles were pooled to examine the effect of reproductive stage at time of treatment. Arcsine square root transformations were used for analyses to satisfy assumptions of normality based on the highest Shapiro-Wilk statistic. Back-transformed values are presented. Columns within the same year with the same letter are not significantly different (*P* ≥ 0.05); lower case: 2010; upper case: 2011.
Figure 4.2. Mean percentage of defoliation (± 95% CI) from field cage studies of adult *C. trifurcata* during pod development of soybeans in Ridgetown, ON, 2010-11. Treatments were 0, 2, 4, 8, 16 beetles per plant and an uncaged control. Timing based on reproductive stage were pooled to examine the effect of number of beetles. Arcsine square root transformations were used for analyses to satisfy assumptions of normality based on the highest Shapiro-Wilk statistic. Back-transformed values are presented. Columns within the same year with the same letter are not significantly different (*P* ≥ 0.05); lower case: 2010; upper case: 2011.
Figure 4.3. Mean percentage of pods with feeding damage (A), number of lesions per pod (B), number of lesions per plant (C), and number of stem lesions per plant (D) from *C. trifurcata* field cages studies during soybean reproductive stages (R3-R6) in Ridgetown, Ontario, Canada, 2010. To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, percentage of pods with feeding damage, number of lesions per pod and number of pod lesions per plant were arcsine square root transformed, and number of lesions per stem was natural log transformed for the analyses. Means presented here are back-transformed to the original scale.
Figure 4.4. Mean of percentage of pods with feeding damage (A), number of lesions per pod (B), number of lesions per plant (C), and number of clipped pods per plant (D) from *C. trifurcata* field cage studies during soybean reproductive stages (R3-R6) in Ridgetown, Ontario, Canada, 2011. To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, number of lesions per pod were square root transformed, number of clipped pods per plant were arcsine square root transformed, and percentage of pods with feeding damage and number of pod lesions per plant were natural log transformed for the analyses. Means presented here are back-transformed to the original scale.
clipped pods per plant in 2011 ($F = 5.49$, df = 12,57, $P < 0.0001$) (Fig. 4.4) with high beetle numbers causing greater damage at later than earlier R-stages. In 2011, 27.4% pod damage occurred at 16 beetles per plant during R6, which nearly doubled from the same treatment in R5. In both years, during R6 infestations, 8 beetles per plant resulted in over 8% pods with feeding. In 2011, during R6, the number of lesions per pod and number of lesions per plant at 16 beetles per plant was 4.7 and 4.3 times that of the 8 beetles per plant, respectively. In 2010, the number of clipped pods per plant increased 2.5 times at 16 compared with 8 beetles per plant. Overall, in 2010, pod-feeding increased at R4 (Fig. 4.3), however, in 2011 there was little pod-feeding until R5 (Fig. 4.4). Stem feeding occurred during R3 and R4 in 2010, but the extent of stem lesions more than doubled during R5 and R6 (Fig. 4.3). In 2011, stem feeding was greatest at R6 ($F = 15.06$, df = 3,57, $P < 0.0001$) and at 16 beetles per plant ($F = 43.59$, df = 4,57, $P < 0.0001$) (Fig. 4.5). When caged and uncaged controls were removed from the analysis, a similar trend was observed for R-stage ($F = 7.20$, df = 3,57, $P = 0.0005$). Overall, there was less stem feeding in 2011 than 2010.

4.1.1 Harvest and Yield

In 2010, there was no significant effect of number of beetles on yield, but yield significantly decreased with increasing R-stage ($F = 5.26$, df = 3,9, $P = 0.0227$) (Fig. 4.6). When caged and uncaged controls were excluded from the analysis a similar trend was observed ($F = 4.39$, df = 3,9, $P = 0.0365$). In 2011, there was no significant effect of R-stage, but caged and uncaged controls had significantly higher yields than the 16 beetles per plant treatment ($F = 3.07$, df = 4,48, $P = 0.0249$) (Fig. 4.7). In both years, there was no significant number of beetles per plant by R-stage interaction effect on yield.

Regressions for yield reductions in 2010 against number of beetles per plant and number
Figure 4.5. Mean (± 95% CI) number of stem lesions per plant based on (A) soybean reproductive stage and (B) number of beetles per plant from *C. trifurcata* field cage studies during soybean reproductive stages in Ridgetown, Ontario, Canada, 2011. To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, number of stem lesions per plant were natural log transformed for the analysis. Means presented here are back-transformed to the original scale.
Figure 4.6. Mean (± SE) yield after field cage studies of adult *C. trifurcata* during pod development of soybean reproductive stages in Ridgetown, ON, 2010-11. Treatments with different numbers of beetles were pooled to examine the effect of reproductive stage at time of treatment. Columns within the same year with the same letter are not significantly different (*P* ≥ 0.05); lower case: 2010; upper case: 2011.
Figure 4.7. Mean (± SE) yield from field cage studies of adult *C. trifurcata* during pod development of soybeans in Ridgetown, ON, 2010-11. Treatments were 0, 2, 4, 8, 16 beetles per plant and an uncaged control. Timing based on reproductive stage were pooled to examine the effect of number of beetles. Columns within the same year with the same letter are not significantly different (*P* ≥ 0.05); lower case: 2010; upper case: 2011.
of feeding days indicated no relationship. In 2011, there was a linear relationship between yield reduction and number of beetles per plant (Fig. 4.8), but not with number of feeding days. In 2011, yield decreased by 1.24% for every additional beetle per plant (Fig. 4.8), but little variance was explained by this model ($R^2 = 0.081$).

In both years, there was no significant interaction between number of beetles per plant and R-stage for 100-seed weight or percentage of oil and protein content ($P > 0.05$). A significant reduction in 100-seed weight was seen in treatments infested at the R6 stage in both years (2010: $F = 6.08$, df = 3,57, $P = 0.0012$; 2011: $F = 2.93$, df = 3,57, $P = 0.0413$) (Table 4.1). When caged and uncaged controls were removed from the analysis, a similar trend was observed (2010: $F = 63.24$, df = 3,41, $P = 0.0316$; 2011: $F = 3.40$, df = 3,41, $P = 0.0264$). Additionally, there was a significant linear relationship ($P < 0.01$) between 100-seed weight and percentage of defoliation for both years (Fig. 4.9). For every 1% increase in percentage of defoliation, there was a decrease of 0.32 and 0.05 g in 100-seed weight in 2010 and 2011, respectively, although this model explained little variance (Fig. 4.9). In 2010, percentage of oil content was highest when feeding occurred during the R4 stage ($F = 3.84$, df = 3,57, $P = 0.0143$) and percentage of protein content was highest when feeding occurred during R3 and R6 stages ($F = 3.12$, df = 3,57, $P = 0.033$) (Table 4.1). When the caged and uncaged controls were removed from the analysis, a similar trend was observed for oil ($F = 4.36$, df = 3,41, $P = 0.0094$) and protein ($F = 2.93$, df = 3,41, $P = 0.0448$) content. In 2011, there was no significant difference in oil or protein content among treatments or R-stages (Table 4.1).

4.1.2 Quality Analysis

In 2010, there was a significant relationship between percentage of total seed damaged and both number of beetles per plant and number of feeding days ($P < 0.01$), although little
Figure 4.8. Linear regression of yield reductions (%) from *C. trifurcata* field cage studies during reproductive soybean stages (R3-R6) in 2011, Ridgetown, ON, Canada. Yield reductions were calculated as a percentage reduction from the caged control for each replicate in a split-plot design, with R-stage as the main effect and number of beetles (0, 4, 8, 16 and uncaged control) per plant as the sub-plot effect.
Table 4.1. Mean (±SE) 100-seed weight, percentage of oil and protein content across all levels of beetle treatments (0, 2, 4, 8, 16 beetles per plant and an uncaged control) from *C. trifurcata* field cage studies during soybean pod-formation in Ridgetown, ON, Canada, 2010-11.

<table>
<thead>
<tr>
<th>Soybean Reproductive Stage</th>
<th>100-Seed Weight (g)</th>
<th>% Oil</th>
<th>% Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3</td>
<td>19.6 ± 0.86 a</td>
<td>15.5 ± 0.44 a</td>
<td>21.7 ± 0.16 bc</td>
</tr>
<tr>
<td>R4</td>
<td>19.3 ± 0.86 a</td>
<td>15.7 ± 0.44 a</td>
<td>22.1 ± 0.16 a</td>
</tr>
<tr>
<td>R5</td>
<td>18.6 ± 0.86 a</td>
<td>15.3 ± 0.44 ab</td>
<td>22.0 ± 0.16 ab</td>
</tr>
<tr>
<td>R6</td>
<td>17.0 ± 0.86 b</td>
<td>14.6 ± 0.44 b</td>
<td>21.6 ± 0.16 c</td>
</tr>
</tbody>
</table>

LS Means within columns followed by the same letter are not significantly different (*P* ≥ 0.05).
Figure 4.9. Linear regression of 100-seed weight and percentage of defoliation from *C. trifurcata* field cage studies (2010: 0, 2, 4, 8 beetles per plant and an uncaged control; 2011: 0, 4, 8, 16 beetles per plant and an uncaged control) during reproductive soybean stages (R3-R6) in 2011, Ridgetown, ON, Canada.
variance was explained by these models ($R^2 = 0.105$ and 0.099, respectively) (Table 4.2). There were similar results in 2011, for percentage of damaged seed in relation to number of beetles per plant and number of feeding days and these models accounted for greater variance ($R^2 = 0.298$ and 0.307, respectively) (Table 4.2). Number of beetles per plant explained more variance than number of feeding days for insect damage, stained brown and stained black seed in 2011 and for damaged seed in 2010 (Table 4.2). Number of feeding days explained more variance for damaged, mouldy dark, mottled brown and shriveled seed in 2011. All regressions had a positive relationship between damaged seed and number of beetles per plant or number of feeding days. Regression analyses indicated no relationship among all other damage categories and number of beetles per plant or number of feeding days ($P > 0.05$) in 2010. A significant interaction was found in 2010 between R-stage and number of beetles per plant for percentage of immature seed ($F = 2.38$, df = 12,57, $P = 0.0144$); however, no obvious directional trends were observed in this interaction.

In 2011, there was a significant interaction between the number of beetles per plant and R-stage for percentage of mouldy white ($F = 2.27$, df = 12,57, $P = 0.0195$) and shrivelled seed ($F = 2.00$, df = 12,57, $P = 0.0414$) (Fig. 4.10). There was a decline in mouldy white seed from 8 to 16 beetles feeding at R4 stage, whereas it increased for all other R-stages. In 2011, the percentage of damaged, insect damaged, immature, mouldy dark, stained brown, stained black, and shrivelled seed increased significantly with number of beetles (Table 4.3 and 4.4). Percentage of mottled brown seed was significantly higher with 16, 8 and 0 beetles per plant than the other treatments in 2011 (Table 4.4). Cage effects were revealed in 2011 for percentage of damaged, mouldy white, mouldy dark, mottled brown and shrivelled seed, where the caged control had significantly higher damage than the uncaged control. Regardless of the number of
Table 4.2. Regression coefficients for number of beetles per plant and number of feeding days against harvested seed quality grading categories from *C. trifurcata* field cage studies with R-stage treatments combined during soybean pod formation in Ridgetown, ON, Canada, 2010-11.

<table>
<thead>
<tr>
<th>Year</th>
<th>Grading Category</th>
<th>Transformation</th>
<th>Slope (b) (x10^-2) ± SE</th>
<th>Number of beetles</th>
<th>Y-intercept (a) ± SE</th>
<th>R²</th>
<th>P-value</th>
<th>Number of feeding days</th>
<th>Slope (b) (x10^-3) ± SE</th>
<th>Y-intercept (a) ± SE</th>
<th>R²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>DMG</td>
<td>arcsine square root</td>
<td>0.87 ± 0.288</td>
<td>0.2025 ± 0.01181</td>
<td>0.11</td>
<td>0.0033</td>
<td>0.58 ± 0.200</td>
<td>0.2044 ± 0.01159</td>
<td>0.10</td>
<td>0.0046</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>DMG</td>
<td>square root</td>
<td>13.77 ± 2.687</td>
<td>4.1317 ± 0.24629</td>
<td>0.30</td>
<td>&lt;0.0001</td>
<td>7.38 ± 1.407</td>
<td>4.3859 ± 0.20800</td>
<td>0.31</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IDMG</td>
<td>arcsine square root</td>
<td>1.13 ± 0.117</td>
<td>0.1005 ± 0.00957</td>
<td>0.55</td>
<td>&lt;0.0001</td>
<td>0.53 ± 0.076</td>
<td>0.1234 ± 0.01004</td>
<td>0.38</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>natural log</td>
<td>3.89 ± 0.915</td>
<td>0.2367 ± 0.07501</td>
<td>0.19</td>
<td>&lt;0.0001</td>
<td>2.22 ± 0.507</td>
<td>0.2838 ± 0.06700</td>
<td>0.20</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MLDYD</td>
<td>natural log</td>
<td>3.00 ± 1.373</td>
<td>1.7234 ± 0.12582</td>
<td>0.07</td>
<td>0.0329</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>STNDBR</td>
<td>natural log</td>
<td>5.66 ± 0.952</td>
<td>1.0357 ± 0.07806</td>
<td>0.31</td>
<td>&lt;0.0001</td>
<td>2.88 ± 0.550</td>
<td>1.3129 ± 0.07273</td>
<td>0.26</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>STNDBL</td>
<td>square root</td>
<td>6.56 ± 1.172</td>
<td>1.6412 ± 0.09604</td>
<td>0.29</td>
<td>&lt;0.0001</td>
<td>3.60 ± 0.657</td>
<td>1.7320 ± 0.08683</td>
<td>0.28</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOTTBR</td>
<td>natural log</td>
<td>3.73 ± 1.410</td>
<td>0.7119 ± 0.12924</td>
<td>0.10</td>
<td>0.0103</td>
<td>2.65 ± 0.708</td>
<td>0.7183 ± 0.10475</td>
<td>0.18</td>
<td>0.0004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Seed grading categories are as follows: DMG=total damaged, IDMG = insect damage, IM=immature, MLDYD=mouldy dark, STNDBR=stained brown, STNDBL=stained black, MOTTBR= mottled brown, SHRVL=shriveled. Data were transformed as indicated for regression analysis in order to achieve linearity or meet assumptions of parametric analysis. All regression coefficients represent transformed parameters.
Figure 4.10. Percentage of (A) mouldy white and (B) shriveled seed from *C. trifurcata* field cage studies in Ridgetown, ON, Canada, 2011. A significant interaction was observed between the number of beetles per plant and soybean reproductive stage. Percentage of mouldy white seed was arcsine square root transformed and shriveled seed was log transformed for analysis. Back-transformed means are presented.
Table 4.3. Mean percentage of seed (± 95% CI) in graded quality categories after *C. trifurcata* field cage studies during soybean pod-formation (R3-R6) in Ridgetown, ON, Canada, 2011.

<table>
<thead>
<tr>
<th>Number of Beetles Per Plant</th>
<th>Damaged (Mean % ± 95% Confidence Interval)</th>
<th>Insect Damaged (Mean % ± 95% Confidence Interval)</th>
<th>Immature (Mean % ± 95% Confidence Interval)</th>
<th>Mouldy White (Mean % ± 95% Confidence Interval)</th>
<th>Mouldy Dark (Mean % ± 95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.1 (+2.48/-1.91) b</td>
<td>0.0 (+0.03/-0.01) a</td>
<td>1.3 (+1.50/-0.90) bc</td>
<td>0.0 (+0.02/-0.02) a</td>
<td>0.4 (+0.36/-0.29) a</td>
</tr>
<tr>
<td>0 uncaged</td>
<td>4.0 (+2.46/-1.89) b</td>
<td>0.0 (+0.04/-0.02) a</td>
<td>1.0 (+1.30/-0.78) c</td>
<td>0.0 (+0.02/-0.02) a</td>
<td>0.2 (+0.31/-0.24) a</td>
</tr>
<tr>
<td>2</td>
<td>5.5 (+2.81/-2.26) b</td>
<td>0.1 (+0.06/-0.04) a</td>
<td>1.9 (+1.90/-1.14) ab</td>
<td>0.0 (+0.02/-0.02) a</td>
<td>0.4 (+0.36/-0.28) a</td>
</tr>
<tr>
<td>4</td>
<td>4.4 (+2.56/-1.99) b</td>
<td>0.0 (+0.04/-0.02) a</td>
<td>1.3 (+1.53/-0.92) bc</td>
<td>0.0 (+0.02/-0.02) a</td>
<td>0.3 (+0.32/-0.25) a</td>
</tr>
<tr>
<td>8</td>
<td>7.7 (+3.21/-2.70) a</td>
<td>0.0 (+0.04/-0.02) a</td>
<td>2.6 (+2.38/-1.43) a</td>
<td>0.0 (+0.02/-0.02) a</td>
<td>0.6 (+0.40/-0.32) a</td>
</tr>
<tr>
<td>0 uncaged</td>
<td>17.5 (+5.43/-4.70) c</td>
<td>0.8 (+0.64/-0.45) c</td>
<td>0.2 (+0.40/-0.31) bc</td>
<td>4.6 (+2.04/-1.68) a</td>
<td>3.7 (+1.80/-1.30) b</td>
</tr>
<tr>
<td>0 uncaged</td>
<td>10.7 (+4.32/-3.59) d</td>
<td>0.7 (+0.60/-0.41) c</td>
<td>0.1 (+0.33/-0.26) c</td>
<td>1.9 (+1.40/-1.02) b</td>
<td>1.7 (+1.05/-0.76) c</td>
</tr>
<tr>
<td>4</td>
<td>20.8 (+5.89/-5.16) bc</td>
<td>2.6 (+1.08/-0.90) b</td>
<td>0.8 (+0.57/-0.44) a</td>
<td>4.1 (+1.92/-1.57) a</td>
<td>6.5 (+2.86/-2.07) a</td>
</tr>
<tr>
<td>8</td>
<td>28.3 (+6.81/-6.07) b</td>
<td>5.1 (+1.44/-1.27) a</td>
<td>0.7 (+0.52/-0.40) ab</td>
<td>5.0 (+2.11/-1.76) a</td>
<td>6.9 (+3.05/-2.20) a</td>
</tr>
<tr>
<td>16</td>
<td>40.0 (+8.02/-7.29) a</td>
<td>6.6 (+1.62/-1.45) a</td>
<td>1.3 (+0.71/-0.54) a</td>
<td>5.8 (+2.25/-1.90) a</td>
<td>7.2 (+3.16/-2.28) a</td>
</tr>
</tbody>
</table>

LS Means within columns followed by the same letter are not significantly different (*P* ≥ 0.05). To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, these data were transformed: 2011 damaged seed were square root transformed; 2010 damaged and insect damaged seed and 2011 insect damaged and immature seed were arcsine square root transformed; 2010 immature, mouldy white, and mouldy dark seed and 2011 mouldy white and mouldy dark seed were natural log transformed.
Table 4.4. Mean percentage of seed (± 95% CI) in graded quality categories after *C. trifurcata* cage infestations during soybean pod-formation (R3-R6) in Ridgetown, ON, Canada, 2011.

<table>
<thead>
<tr>
<th>Number of Beetles Per Plant</th>
<th>Mean % Seed (± 95% Confidence Interval)</th>
<th>Stained Brown</th>
<th>Stained Black</th>
<th>Mottled Purple</th>
<th>Mottled Brown</th>
<th>Shriveled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2010</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.3 (+0.20/-0.18) a</td>
<td>0.2 (+0.12/-0.09) a</td>
<td>0.3 (+0.26/-0.18) a</td>
<td>0.0 (+0.09/-0.04) a</td>
<td>0.7 (+0.75/-0.52) a</td>
<td></td>
</tr>
<tr>
<td>0 uncaged</td>
<td>0.4 (+0.21/-0.19) a</td>
<td>0.1 (+0.10/-0.06) a</td>
<td>0.5 (+0.31/-0.23) a</td>
<td>0.1 (+0.10/-0.06) a</td>
<td>0.8 (+0.84/-0.58) a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.6 (+0.24/-0.21) a</td>
<td>0.1 (+0.09/-0.05) a</td>
<td>0.3 (+0.26/-0.18) a</td>
<td>0.2 (+0.16/-0.11) a</td>
<td>0.7 (+0.78/-0.53) a</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.4 (+0.22/-0.19) a</td>
<td>0.1 (+0.10/-0.07) a</td>
<td>0.4 (+0.30/-0.22) a</td>
<td>0.2 (+0.14/-0.10) a</td>
<td>0.9 (+0.86/-0.59) a</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.5 (+0.22/-0.19) a</td>
<td>0.1 (+0.12/-0.08) a</td>
<td>0.3 (+0.25/-0.17) a</td>
<td>0.1 (+0.12/-0.07) a</td>
<td>1.1 (+0.94/-0.65) a</td>
<td></td>
</tr>
<tr>
<td><strong>2011</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.9 (+0.97/-0.73) c</td>
<td>2.6 (+1.22/-1.02) b</td>
<td>0.7 (+0.50/-0.37) a</td>
<td>1.7 (+1.00/-0.73) a</td>
<td>3.0 (+1.29/-0.97) c</td>
<td></td>
</tr>
<tr>
<td>0 uncaged</td>
<td>1.9 (+0.97/-0.73) c</td>
<td>1.8 (+1.05/-0.86) b</td>
<td>0.5 (+0.44/-0.31) a</td>
<td>0.7 (+0.63/-0.46) b</td>
<td>1.4 (+0.79/-0.60) d</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.2 (+1.07/-0.80) bc</td>
<td>2.6 (+1.22/-1.02) b</td>
<td>0.3 (+0.35/-0.21) a</td>
<td>0.6 (+0.58/-0.43) b</td>
<td>3.6 (+1.51/-1.14) bc</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.4 (+1.46/-1.09) b</td>
<td>5.1 (+1.59/-1.39) a</td>
<td>0.5 (+0.42/-0.29) a</td>
<td>1.7 (+1.01/-0.74) a</td>
<td>4.9 (+1.94/-1.46) ab</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>6.2 (+2.38/-1.79) a</td>
<td>6.4 (+1.76/-1.56) a</td>
<td>0.5 (+0.42/-0.29) a</td>
<td>3.1 (+1.49/-1.09) a</td>
<td>6.5 (+2.44/-1.84) a</td>
<td></td>
</tr>
</tbody>
</table>

LS Means within columns followed by the same letter are not significantly different ($P \geq 0.05$). To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, these data were transformed: 2010 stained black, mottled purple and mottled brown seed and 2011 mottled purple seed were arcsine square root transformed; 2010 stained brown and shriveled seed and 2011 stained brown, mottled brown and shriveled seed were natural log transformed.
beetles, mottled purple seeds were lowest during R6 infestations for both years (Table 4.5).

When caged and uncaged controls were removed from analysis, results for percentage of mottled purple seed had the same trend for 2011 but R-stage was not significant in 2010 ($F = 2.13, df = 3.41, P = 0.1109$; 2011: $F = 4.12, df = 3.41, P = 0.0121$). In 2010, the highest levels of mouldy dark and stained black seed occurred in the R5 infestations, although both categories were relatively low (Table 4.5). There was no significant R-stage effect when caged and uncaged controls were removed from the analysis for mouldy dark ($F = 1.96, df = 3.41, P = 0.1343$) and stained black ($F = 2.63, df = 3.41, P = 0.0628$) seed.

Only three out of 176 samples over the two years of study tested positive for BPMV by ELISA. Plots that tested positive for BPMV had 2 and 4 beetles per plant during R6 infestations in 2010, and one of the naturally infested plots in 2011. Consistent with these results, very few BPMV symptoms were observed in the field.

4.1.3 Economic Injury Level

There was no relationship between yield and number of beetles per plant when years were analyzed separately. When years were combined, there was a significant relationship between yield and number of beetles per plant ($R^2=0.104, P=0.0002$) (Fig. 4.11), therefore, these results were used for EIL calculations. For every adult *C. trifurcata* increase, yield was reduced by 59.8 kg/ha (0.89 bu/ac). EILs ranged from 0.34 to 2.50 beetles per plant, or 10 to 75 beetles per m of row (Table 4.6). Economic thresholds (ETs) are typically set at 80% of EILs (Pedigo and Rice 2009). ETs determined by this study during soybean reproductive stages (R3-R6) ranged from 0.27 to 2.00 beetles per plant, or 8 to 60 beetles per m of row. The range of soybean market values can be viewed as a continuum with non-IP varieties in the lower two thirds and IP varieties in the upper two thirds, and market values for both categories may overlap towards the middle (Table 4.6).
Table 4.5. Mean percentage of seed (± 95% CI) with a significant effect from soybean reproductive stage (R3-R6) during field cage studies of *C. trifurcata* in Ridgetown, ON 2010-2011.

<table>
<thead>
<tr>
<th>Soybean Reproductive Stage</th>
<th>Mouldy Dark 2010</th>
<th>Stained Black 2010</th>
<th>Mottled Purple 2010</th>
<th>Mottled Purple 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3 0.51 (+0.361/-0.291) a</td>
<td>0.04 (+0.063/-0.035) b</td>
<td>0.84 (+0.374/-0.306) a</td>
<td>0.44 (+0.386/-0.266) ab</td>
<td></td>
</tr>
<tr>
<td>R4 0.32 (+0.315/-0.254) ab</td>
<td>0.08 (+0.080/-0.051) b</td>
<td>0.34 (+0.252/-0.182) b</td>
<td>0.74 (+0.481/-0.362) a</td>
<td></td>
</tr>
<tr>
<td>R5 0.55 (+0.370/-0.299) a</td>
<td>0.20 (+0.121/-0.093) a</td>
<td>0.21 (+0.206/-0.137) b</td>
<td>0.62 (+0.447/-0.327) a</td>
<td></td>
</tr>
<tr>
<td>R6 0.14 (+0.272/-0.219) b</td>
<td>0.14 (+0.102/-0.074) ab</td>
<td>0.14 (+0.176/-0.106) b</td>
<td>0.24 (+0.300/-0.179) b</td>
<td></td>
</tr>
</tbody>
</table>

LS Means within columns followed by the same letter are not significantly different (*P* ≥ 0.05). To satisfy the assumptions of normality based on the highest Shapiro-Wilk statistic, these data were transformed: 2010 stained black and mottled purple and 2011 mottled purple seed were arcsine square root transformed; 2010 mouldy dark were natural log transformed.
Figure 4.11. Linear regression of yield and number of beetles per plant from *C. trifurcata* field cage studies (2010: 0, 2, 4, 8 beetles per plant; 2011: 0, 4, 8, 16 beetles per plant) during reproductive soybean stages (R3-R6) in 2011, Ridgetown, ON, Canada.

\[
y = 2441.2 - 59.77x \\
R^2 = 0.104 \\
P = 0.0002
\]
Table 4.6. Economic injury levels (i.e. number of beetles per plant or metre of row) for *C. trifurcata* during pod-formation on soybeans for a range of soybean market values and pest management costs.

<table>
<thead>
<tr>
<th>Soybean market values</th>
<th>Pest management costs $/ha ($/ac)</th>
<th>15.00 (6.07)</th>
<th>25.00 (10.12)</th>
<th>35.00 (14.16)</th>
<th>45.00 (18.21)</th>
<th>55.00 (22.26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$/kg $/bu</td>
<td>Beetles per plant</td>
<td>Beetles per metre row*</td>
<td>Beetles per plant</td>
<td>Beetles per metre row*</td>
<td>Beetles per plant</td>
<td>Beetles per metre row*</td>
</tr>
<tr>
<td>0.37 10.00</td>
<td>0.68 20.5</td>
<td>1.14 34.1</td>
<td>1.59 47.79</td>
<td>2.05 61.4</td>
<td>2.50 75.1</td>
<td></td>
</tr>
<tr>
<td>0.40 11.00</td>
<td>0.62 18.6</td>
<td>1.03 31.0</td>
<td>1.45 43.44</td>
<td>1.86 55.9</td>
<td>2.28 68.3</td>
<td></td>
</tr>
<tr>
<td>0.44 12.00</td>
<td>0.57 17.1</td>
<td>0.95 28.4</td>
<td>1.33 39.82</td>
<td>1.71 51.2</td>
<td>2.09 62.6</td>
<td></td>
</tr>
<tr>
<td>0.48 13.00</td>
<td>0.53 15.8</td>
<td>0.88 26.3</td>
<td>1.23 36.76</td>
<td>1.58 47.3</td>
<td>1.93 57.8</td>
<td></td>
</tr>
<tr>
<td>0.51 14.00</td>
<td>0.49 14.6</td>
<td>0.81 24.4</td>
<td>1.14 34.13</td>
<td>1.46 43.9</td>
<td>1.79 53.6</td>
<td></td>
</tr>
<tr>
<td>0.55 15.00</td>
<td>0.46 13.7</td>
<td>0.76 22.8</td>
<td>1.06 31.86</td>
<td>1.37 41.0</td>
<td>1.67 50.1</td>
<td></td>
</tr>
<tr>
<td>0.59 16.00</td>
<td>0.43 12.8</td>
<td>0.71 21.3</td>
<td>1.00 29.87</td>
<td>1.28 38.4</td>
<td>1.56 46.9</td>
<td></td>
</tr>
<tr>
<td>0.62 17.00</td>
<td>0.40 12.0</td>
<td>0.67 20.1</td>
<td>0.94 28.11</td>
<td>1.20 36.1</td>
<td>1.47 44.2</td>
<td></td>
</tr>
<tr>
<td>0.66 18.00</td>
<td>0.38 11.4</td>
<td>0.63 19.0</td>
<td>0.88 26.55</td>
<td>1.14 34.1</td>
<td>1.39 41.7</td>
<td></td>
</tr>
<tr>
<td>0.70 19.00</td>
<td>0.36 10.8</td>
<td>0.60 18.0</td>
<td>0.84 25.15</td>
<td>1.08 32.3</td>
<td>1.32 39.5</td>
<td></td>
</tr>
<tr>
<td>0.73 20.00</td>
<td>0.34 10.2</td>
<td>0.57 17.1</td>
<td>0.80 23.89</td>
<td>1.02 30.7</td>
<td>1.25 37.5</td>
<td></td>
</tr>
</tbody>
</table>

* Values represent the number of adults per metre of row, with 0.76 m row spacing, seeding rates of 400,000 seeds/ha, and a plant stand of 30 plants per metre of row (Ontario Ministry of Agriculture Food and Rural Affairs 2011a).
4.2 Discussion

In 2010, defoliation was lowest during R3 which was unexpected given that the leaves were still very green and the canopy was full. The decrease in defoliation over R-stage observed in 2011 may be superficial because leaves yellow and drop as soybeans start senescing in the later reproductive stages, therefore, fewer leaves are available for feeding and those that remain may be less attractive. The interactions between number of beetles per plant and R-stage suggest that pod- and stem-feeding increase with number of beetles per plant and with pod development. In 2011, during R6, doubling of beetles from 8 to 16 resulted in over 4-fold increases in the number of lesions per pod and number of lesions per plant. This could possibly be related to physiological changes that increase feeding behaviour as beetles prepared to overwinter.

Overall, pod-feeding increased earlier in 2010 during R4, compared with an increase in 2011 at R5. This increase in pod-feeding during later stages of reproduction may be partially due to the beginning of leaf senescence, or increasing palatability of pods with seed development. Despite efforts to keep control plots free of insects, pod damage was observed in the caged controls during the R6 infestation with rates of 0.5% damaged pods in both 2010 and 2011; although this was still significantly lower than the cages with beetles.

The reduction in yield with increasing R-stage in 2010 may be explained by the plots being thinned prior to beetle placement in cages; therefore, R3 plots would have had a greater possibility to compensate for the extra space, thereby increasing plant size and pod set.

Regression models for yield reduction explained very little variance, perhaps a result of the small plot size (5 plants), where large yield differences are more difficult to quantify. However, the reduction in 100-seed weight in 2010 is unlikely to be a result of thinning plots later, because
although yield may have been affected, this should not affect seed size. However, reduced seed size may have been a result of increased pod-feeding.

In general, there were greater seed quality issues in 2011 than 2010. In 2011, there was higher weed pressure, poorer soil and an overall shorter stand in the field than in 2010, but these do not likely explain presence of pathogens. It was surprising to find higher mottled brown seed with 0 than 4 beetles because that is one of the symptoms of BPMV; however, this symptom is not exclusive to this virus. Soybean mosaic virus (SMV) is also known to cause mottled seed (Ross 1968, 1969, Giesler et al. 2002) and although control plots were sprayed with insecticides, A. glycines were observed in the field. Without testing for SMV, it is difficult to say with certainty what caused the mottling.

A higher level of defoliation was observed in the uncaged controls compared with the caged controls, indicating that C. trifurcata were effectively excluded from the cages. However, there was a higher incidence of percentage of damaged, mouldy white, mouldy dark, mottled brown and shriveled seed in the caged controls compared with the uncaged controls in 2011. This may be a result of microclimatic factors affecting seed quality which are independent of the number of beetles present. Additionally, conditions within cages may have been favourable to other insects, such as A. glycines, which may have affected seed quality.

Results from this study indicate that C. trifurcata feeding during soybean reproductive stages can affect both yield and quality, with more deleterious effects during seed development. Average soybean yield for southern Ontario in 2011 was 3154 kg/ha (Statistics Canada 2011a). According to the yield reduction index regression, for every C. trifurcata adult, yield is reduced by 1.24% or 39 kg/ha (Fig. 4.7); the estimate of yield loss from the yield (kg/ha) regression was 60 kg/ha (Fig. 4.9). The estimates from this study are 40-60 kg/ha lower than previously
determined (Smelser and Pedigo 1992c). One major difference between these studies is that Smelser and Pedigo (1992c) monitored adult mortality after cage infestations. As the yield loss estimates from this study do not account for declining beetle numbers over time, yield loss per beetle may have been underestimated.

Previous studies estimated that *C. trifurcata* adults feed for approximately 21 d (Isely 1930, Smelser and Pedigo 1992b). In the current study, the duration of R6 was 28 d in 2011. If adults ceased feeding after day 21, regressions with number of feeding days may underestimate their yield impact. In addition, there may have been higher mortality during R3 and R4 than later stages, because adults used in these treatments may have been older overwintered adults, rather than newly eclosed adults. However, there were no obvious trends in the data to suggest that beetle mortality was higher at any given stage over the others. In the future it would be useful to monitor beetle mortality to gain more accurate estimates of feeding during reproductive stages.

Based on *C. trifurcata* impact on yield, EILs for IP soybeans would be about lower than for non-IP soybeans, due to their greater value. Because quality is of utmost importance to IP growers, EILs may need to be reduced even further to avoid effects on quality. The lowest estimated EIL was 0.34 beetles per plant (Table 4.6) and as feeding assessments and quality analysis indicate, even treatments sprayed weekly could experience insect damage that results in unacceptable levels of damaged seed. Current economic thresholds for *C. trifurcata* in Ontario are 15% defoliation for R3 and R4, and 25% defoliation during R5 and R6 stages (unless pod-feeding is observed) (Ontario Ministry of Agriculture Food and Rural Affairs 2011b). For IP food grade and seed soybeans, the current threshold is 10% pods with feeding damage and active adults in the field (Ontario Ministry of Agriculture Food and Rural Affairs 2011b). These
thresholds are based on the assumption that there are two summer generations; however, only one generation occurs in southwestern Ontario (Chapter 3). Thresholds for late-season pod-feeding for Ontario growers should be updated based on the EILs established in this study.

Recommendations from this study begin with choosing the appropriate ET, which should be chosen based on management costs, expected yields and estimated market value. Producers should monitor *C. trifurcata* for increasing populations during soybean reproductive stages. When ETs are reached, proper control action may include a foliar insecticide application.
C. trifurcata is a relatively new and significant pest in the production of soybean in Ontario. The goal of this thesis was to assess whether there were any unique impacts of this pest on soybean production in Ontario in terms of virus transmission, or yield and quality, and to provide integrated pest management (IPM) tools in response to this new range expansion. The objectives of this thesis were to determine the phenology of C. trifurcata in Ontario, including life cycle and degree day requirements; to evaluate the impact of late-season pod-feeding on soybean yield and quality; to assess the extent of virus transmission; and finally, to develop an economic threshold for late-season pod-feeding and appropriate management recommendations in the context of the current agronomic practices of Ontario soybean producers.

Phenology: Life Cycle and Degree Day Requirements

C. trifurcata underwent only one summer generation in Ontario, as demonstrated by recovery of only one complete cycle of eggs, larvae and pupae from soil samples in the phenology study. The degree day model for development from egg deposit to adult emergence, based on the temperature-dependent development laboratory study was not different from what was observed in the field, predicting 581 ± 39.7 DD (base 10.25°C) and 589 ± 66.8 DD (base 10.25°C), in laboratory and field, respectively. The best estimate of lower developmental threshold was 10.2 ± 0.38°C. The degree day model developed in the laboratory agreed with the data obtained in the field; however, the degree day requirements and lower thresholds were based on laboratory-reared C. trifurcata which were field-collected and adapted to southwestern
Ontario. Therefore, when attempting to apply these models in a different locale, the current model should be validated first because differences noted between the model developed in this study and those reported elsewhere indicate this model may fit better in the northern part of the range of *C. trifurcata* distribution.

Extremely low survivorship in the temperature-dependent development study, at 18.8 and 30.9°C, limited the number of observations at these temperatures. Survivorship was 4-fold greater at temperatures averaging 23.4 and 26.1°C, than at 18.8°C. Since average soil temperatures were between 19.0 and 21.6°C in southwestern Ontario field studies, survivorship of immature *C. trifurcata* may be reduced in its northern range. The prominently southern geographic distribution in North America suggests *C. trifurcata* has a higher tolerance for heat than cold.

There was evidence of a partial second generation at most field sites, similar to findings in eastern South Dakota (Hammack et al. 2010), however, insufficient degree days accumulated to support complete development. Developmental rates increased with temperature along the linear range of the model; therefore, the potential exists, especially during a warmer than usual growing season for a second summer generation of *C. trifurcata* to complete development. In southern Ontario, additional accumulations of ~ 350 DD (base 10.25°C), would likely be sufficient to support a second generation. Furthermore, warming temperatures due to climate change may aid additional range expansion into more northern regions of Ontario. Further research is needed to assess the overwintering success and potential range expansion of *C. trifurcata* to more northern regions in Ontario under future climates.

Where only one summer generation occurs, the overwintered colonizing population may be a good target for control (Witkowski and Echtenkamp 1996, Bradshaw et al. 2008, Piitz...
The use of insecticidal seed treatments, namely thiamethoxam, can reduce colonizing *C. trifurcata* early in the growing season (Witkowski and Echtenkamp 1996, Piitz 2012), thereby, reducing abundance of subsequent generations (Koch et al. 2005). In Ontario, growers rely heavily on seed treatments for early season control, and a noticeable decline in *C. trifurcata* abundance and incidences of economic populations were observed over the two years of this study (personal observation). Increasing and almost sole use of thiamethoxam increases the probability of resistance development due to repeat exposure. Alternatively, late-season pest pressure from *C. trifurcata* adults can be treated with a foliar insecticide application, primarily pyrethroids. While there is currently no evidence of *C. trifurcata* resistance to neonicotinoids (i.e. thiamethoxam) or pyrethroids (i.e. lambda-cyhalothrin) in Ontario, resistance to these chemistries has developed in other insects (Hemingway and Ranson 2000, Gorman et al. 2007). Future research is needed to identify and register chemistries to be used as insecticidal seed treatments in Canada with different modes of action to minimize risk of resistance, as well as the need to develop a baseline for *C. trifurcata* resistance management.

Based on the current study, monitoring *C. trifurcata* in southwestern Ontario soybean fields should begin shortly after soybean emergence until populations decline and begin again when degree days approach 500 DD (base 10.25°C), during pod formation. Thiamethoxam seed treatments can provide early season control, but populations should be monitored for insecticide resistance. If new chemistries are registered for *C. trifurcata* in soybean, they should be incorporated into the IPM and IRM programs by alternating chemistries. New chemistries of seed treatments can be used by alternating years and foliar insecticides can used by alternating years, or alternate applications within years if multiple treatments are required. In the meantime, producers should consider including one year of planting non-insecticide treated seed into their
crop rotation.

**Impact of Late-Season Pod-Feeding**

Pod, peduncle and stem feeding increased as soybeans matured through reproductive stage (R-stage) and as number of beetles increased. Pod-feeding increased dramatically from R5 to R6, while defoliation decreased during these stages. Yield reductions occurred with increasing number of beetles, regardless of R-stage at the time of feeding, and losses approached 60 kg/ha per beetle per plant.

Seed quality, assessed by number of damaged, immature, mouldy, stained and shriveled seed, declined as beetle numbers increased, regardless of R-stage at the time of feeding. Presence of cages, may have had an impact on seed quality, however, that impact could not be quantified in the current study due to limitations of the data collected. Additionally, very few symptoms of bean pod mottle virus were observed in the field, with only three samples over two years of study testing positive for BPMV by enzyme linked immunosorbent assay (ELISA). Future research should control for cage effects on seed quality and on the occurrence of fungal and viral pathogens with emphasis on seed quality categories found to be affected by *C. trifurcata* feeding.

In southern Ontario, abundance of *C. trifurcata* adults is often synchronized well with soybean pod formation. Observed increases in pod-feeding corresponded well to the emergence of summer generation adults and soybean seed formation in the current study. Because the potential for damage is greatest when *C. trifurcata* and crop development are synchronized, monitoring of *C. trifurcata* during R5 and R6 stages is critical. Using the degree day model as a predictor, when degree days approach 500, soybean fields should be sampled for increasing
numbers of adult *C. trifurcata*. Foliar insecticides can be used to reduce economically damaging populations.

**Economic thresholds**

Economic injury levels (EILs) during R-stages (R3 to R6) for *C. trifurcata* in soybean ranged from 0.34 to 2.50 beetles per plant, or 10 to 75 beetles per m of row. Economic thresholds (ETs) are recommended to be 80% of the EIL (Pedigo and Rice 2009), resulting in ETs that range from 0.27 to 2.00 beetles per plant, or 8 to 60 beetles per m of row, applicable for the economics of soybean production during the period of this study. The range of soybean market values used for these estimates represents non-IP varieties and IP varieties as a continuum from lowest to highest values, respectively; however, market values for both categories may overlap towards the middle. Premiums for IP soybean varieties can increase their market value by up to 33%, and consequently the EILs and ETs, may be 18-28% lower for IP versus non-IP soybeans. The ETs could be further refined for IP soybean varieties by evaluating yield impacts on specific classes (i.e. large and small-seed varieties).

In this study, EILs and ETs are based on number of beetles per plant or number of beetles per m of row. Although *in situ* counts can be accurate and efficient during early vegetative stages, sweep-net sampling may provide the best estimate of beetle numbers during later stages of plant growth (Rudd and Jensen 1977). Further research is needed to translate the EILs and ETs from this study to number of beetles per sweep to increase accuracy of monitoring and use of ETs.

Monitoring during soybean pod-formation is crucial for an effective IPM program. Adult populations can be controlled by foliar insecticides (i.e. lambda-cyhalothrin, dimethoate, and
imidacloprid + deltamethrin) (Ontario Ministry of Agriculture Food and Rural Affairs 2011-2012). However, there is evidence in the Mississippi Delta for the development of tolerance to pyrethroids (i.e. lambda-cyhalothrin) in *C. trifurcata* as a result of incidental exposures from applications made for control of other pests (Musser et al. 2012). Soybean pests in Ontario that may require foliar treatments include soybean aphid, *Aphis glycines* Matsumura, green stink bug, *Nezara viridula* (L.), and brown stink bug, *Euschistus servus* (Say). Therefore, a foliar application can be used to reduce beetle numbers when thresholds are reached but, for resistance management, different modes of action should be alternated each year or between applications within a growing season if multiple applications are required or multiple pests are targeted.

**Recommendations and Conclusions**

Management costs, expected yields and estimated market values should be evaluated prior to monitoring and the appropriate ETs should be chosen. Thiamethoxam seed treatments can be used for early season control if populations were high in nearby fields the previous year. Soybean fields should be monitored at emergence for colonizing *C. trifurcata* adults. Producers should consider including one year in their crop rotation for planting non-insecticide treated seed as part of an IRM program. Late-season monitoring programs should begin during early pod-filling stages when degree days approach 500 DD (base 10°C) or 900 (base 50°F) (to detect summer generation adult emergence. If economic thresholds are reached, appropriate control action may include foliar insecticide applications (i.e. lambda-cyhalothrin, dimethoate, or imidacloprid + deltamethrin) in order to prevent or minimize yield and quality losses. Registered chemistries should be alternated each year, or within years if multiple applications are required,
for proactive resistance management.

The goals and objectives of this study were achieved by determining the life cycle, degree day requirements, and impact of late-season pod-feeding by *C. trifurcata* in southwestern Ontario. Until now, no research has been conducted on phenology and degree day requirements of *C. trifurcata* in Ontario. To my knowledge, this was also the first study to evaluate late-season pod-feeding during different soybean reproductive stages in North America. This study has evaluated the life history and impact of *C. trifurcata* in southern Ontario and developed revised recommendations for management of this pest, to help ensure the continued success of Ontario soybean producers.
References


**Bergant, K., and S. Trdan. 2006.** How reliable are thermal constants for insect development when estimated from laboratory experiments? Entomologia Experimentalis et Applicata 120: 251-256.


Subject Books, Guelph, Ontario, Canada.

Bradshaw, J. D., M. E. Rice, and J. H. Hill. 2007. No-choice preference of Cerotoma trifurcata (Coleoptera: Chrysomelidae) to potential host plants of bean pod mottle virus (Comoviridae) in Iowa. J. Econ. Entomol. 100: 808-814.

Bradshaw, J. D., M. E. Rice, and J. H. Hill. 2008. Evaluation of management strategies for bean leaf beetles (Coleoptera: Chrysomelidae) and bean pod mottle virus (Comoviridae) in soybean. J. Econ. Entomol. 101: 1211-1227.


Hammack, L., J. L. Pikul, and M. S. West. 2010. Phenology and abundance of bean leaf beetle (Coleoptera: Chrysomelidae) in eastern South Dakota on alfalfa and soybean relative to tillage, fertilization, and yield. Environ. Entomol. 39: 727-737.


in soybean. Plant Dis. 87: 1221-1225.

Maienfisch, P., H. Huerlimann, A. Rindlisbacher, L. Gsell, H. Dettwiler, J.


869-885.


agricultural insect pests. Agricultural and Forest Meteorology 57: 221-240.


Ruppel, R. F. 1971. An asymmetrical gynandromorph of Cerotoma facialis (Coleoptera: Notariidae)


Smelser, R. B., and L. P. Pedigo. 1992b. Bean leaf beetle (Coleoptera: Chrysomelidae) herbivory on leaf, stem, and pod components of soybean. J. Econ. Entomol. 85: 2408-


Statistics Canada. 2011c. Farm cash receipts - agriculture economic statistics, Catalogue No. 21-011-XWE.


Vijverberg, H. P., J. M. van der Zalm, and J. van den Bercken. 1982. Similar mode of action of pyrethroids and DDT on sodium channel gating in myelinated nerves.


Waldbauer, G. P., and M. Kogan. 1975. Position of bean leaf beetle eggs in soil near soybeans
determined by a refined sampling procedure. Environ. Entomol. 4: 375-380.


